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URINARY GLUCURONIDES IN RHEUMATIC DISEASES

BY

TOIVO HOLOPAINEN

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE MEDICAL CLINIC, UNIVERSITY OF TURKU, CHIEF: PROFESSOR P. BRUMMER, M. D., AND FROM THE DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF TURKU, CHIEF: PROFESSOR K. HARTIALA M. D.

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TURKU 1958

KUOPIO — KIRJAPAINO OY SAVO 1958

to my family.

PREFACE

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Toivo Holopainen

CONTENTS

I. GLUCURONIC ACID AND GLUCURONIDE SYNTHESIS

Glucuronic acid

In 1855 Schmid found euxanthic acid magnesia to constitute the bulk of Indian yellow (Purree) precipitated in the urine of camels that had fed on mango (the fruit of Carcinia mangostana). He regarded euxanthic acid (which is known today to be an euxanthone glucuronide) as a conjugated compound and this observation was probably one of the first of conjugated glucuronic acid. Later, in 1870, Baeyer found that acid hydrolysis of euxanthic acid yielded a substance that reduces copper in alkaline solution. This substance resembled saccharic acid and had the formula $C_6H_{10}0_7$ which is now known to apply to glucuronic acid. Mering and Musculus in 1875 observed that a strongly acid, copper-reducing and levorotatory substance was excreted in the urine following an intake of chloral hydrate. The substance was isolated in crystalline form by these investigators and called urochloralic acid.

In animal urine, following an intake of indole, Baumann (in 1877) found another indole-containing compound besides indoxylsulphuric acid. It combined with an organic radical (now known to be glucuronic acid).

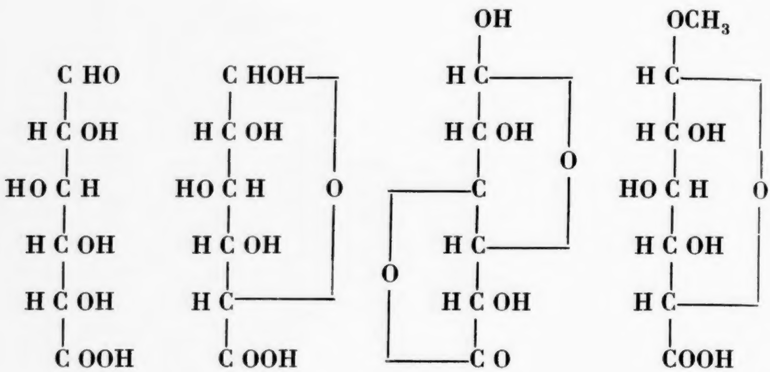
In 1879 Schmiedeberg and Meyer fed dogs on camphor and isolated from the urine α- and β-camphoglycuronic acid, and glucuronic acid (as lactone, as glucurone) which they named "glycuronic acid". They described the stucture of the compound as

$$(CHOH)_4 \begin{matrix} CHO \\ COOH \end{matrix} .$$

The compound was dextrorotatory, unlike Jaffe's (1879) uronitroluol acid which was levorotatory. Schmiedeberg and Meyer

believed glucuronic acid to represent an intermediate in the sugar metabolism of the body the further oxidation of which was prevented by camphor conjugation.

Artz and Osman in 1951 formulated the structure of glucuronic acid as follows:



I II III IV

Fig 1

(I and II represent structures in which glucose has been oxidized at carbon 6 with carbon 1 remaining unchanged; this situation is not likely to occur during a simple oxidation of glucose.) The lactone (III) of glucuronic acid is generally named glucurone. The term glucuronolactone is associated more with the nomenclature of carbohydrate metabolism. "Methyl glucuronide", "Methyl glucuronic acid" and "Methyl glucuronoside" are names that have been employed to designate the compound (IV). The compound (III) has been proved to have a furane form (Reeves E. 1940; Smith F. 1944) while the compounds (II, IV) have been shown to possess a pyranoid structure (Owen L. N., Peat S. and Jones W. J. G. 1941 and Hardegger E. and Spitz D. 1949, 1950).

The mechanism of the glucuronide synthesis in the organism

The liver and to some extent also the kidney have been found to possess a glucuronide-forming ability (Kerr, Graham and Levvy 1948, Levvy and Storey 1949, Karunairatnam, Kerr and Levvy 1949, Storey 1950, Hartiala and Telivuo 1955). Considerable glucuronide synthesis has now been found to occur in the different parts of the alimentary canal such as the stomach, duodenum, ileum and colon (Hartiala 1954, 1955).

3-carbon atom compounds were previously regarded as precursors of the glucuronic acid synthesis (e.g. Lipschitz and Bueding 1939, Martin and Stenzell 1944). Later investigations made with C^{14}-labelled 3-carbon atom compounds (Doerschuk 1952) lent support to this view. However, many investigations carried out in the last few years whish have employed C^{14}-labelled glucose at carbon 1 or 6 have shown the glucose to change into glucuronic acid in an intact chain without prior decomposition (Mosbach and King 1950, Bidder 1952, Eisenberg and Gurin 1952, Douglas and King 1953, Packham and Butler 1952).

The direct oxidation of glucose to glucuronic acid can be conceived of only chemically; the acetalhydroxyl group, being protected, should be oxidized. It has not, however, been possible to prove this experimentally. In the last few years the glucuronide synthesis of several compounds has been reported to occur via the cycle of the uridinphosphate compounds (Ströminger 1955, Isselbacher 1956, Levvy 1956), Fig. 2. In this chain of events adenosine triphosphoric acid (ATP) and uridindiphosphoric acid (UDP) are considered (1) to change into uridintriphosphoric acid (UTP) and into adenosinediphosphoric acid (ADP) in the organism under the influence of nucleoside-diphosphokinase (Berg and Joklik 1953, 1954, Trucco 1951). (2) In the presence of uridindiphosphoglucose-pyrophosphorylase the UTP thus formed and glucose-1-phosphate become uridindiphosphoglucose (UDPG), and phosphorus is liberated (Munch-Petersen, Kalckar, Cutolo and Smith 1954). (3) UDPG is oxidized with diphosphonucleotide (DPN +) to *uridindiphosphoglucuronic acid* (UDPGA) under the influence of UDPG-dehydrogenase (Fig. 2), DPNH and hydrogen originate concurrently (Ströminger, Kalckar, Axelrod and Maxwell 1954). (4) The UDP glucuronic acid

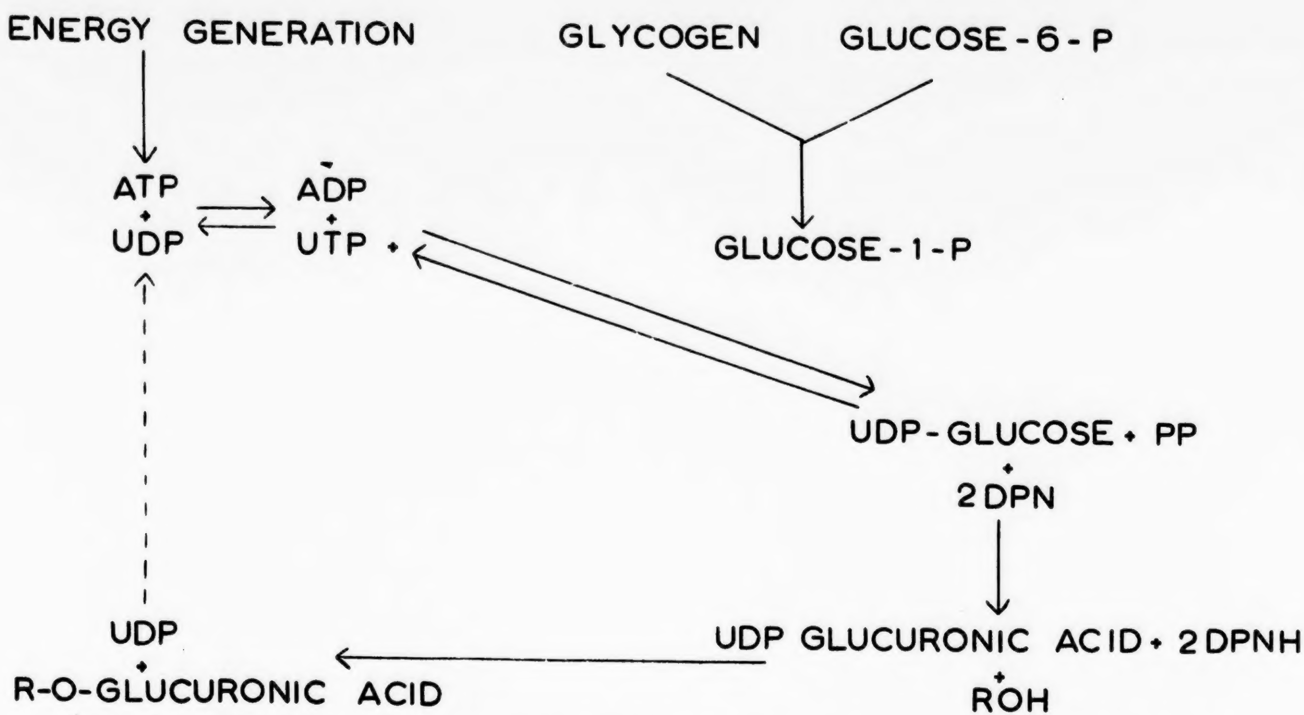


Fig. 2. — The over-all reaction in glucuronide synthesis : a UDP cycle (Ströminger 1955, Isselbacher 1956).

thus formed is the active form of glucuronic acid which forms ester- and ethereal-type glucuronides with several substances in the presence of glucuronosyltransferase in the microtome preparations of liver (Dutton and Storey 1954, Smith and Mills 1954, Isselbacher 1956, Dutton 1956).

β-glucuronidase, a group-specific enzyme, which catalyzes the hydrolysis of β-glucuronides to aglucone and free glucuronic acid must be borne in mind when considering the glucuronide synthesis. Glucuronidase was found to catalyze the conjugation of glucusiduronic acid with alcohol in the presence of glucuronosyltransferase (Fishman and Green 1957). Levvy (1956), on the other hand, decided that β-glucuronidase did not participate in the glucuronide synthesis at any phase of the process.

The information elicited so far is deficient, mostly hypothetical, on the interrelationship of the exogenic glucuronide synthesis and the metabolism of the glucuronic acid-containing compounds of the connective tissue.

Compounds excreted in urine as glucuronides

Glucuronic acid conjugation is the most common of the detoxications found in different animal species. This synthesis results in a reduction in toxicity and a considerably increased solubility at the physiological pH levels (Williams 1947).

Types of glucuronide formation: Two kinds of glucuronides occur in the body, viz. the ethereal and ester types (Williams 1947). The former, e.g. phenyl and methyl glucuronides, are stable against alkaline reagents and do not reduce alkaline copper reagents. Ester glucuronides on the other hand, e.g. benzyl glucuronide, decompose when a mild alkali liberates glucuronic acid and this is followed by a reduction in alkaline cupric oxide. A compound is known which is made up of both these glucuronide types, i.e. p-glucuronoside-benzoylglucuronide. (The compound was isolated from the urine of dogs after feeding them para-hydroxybenzoic acid, Quick 1932).

As regards drugs used for rheumatic diseases, following the administration of different salicylate doses (salts of ortho-(hydr) oxybenzoic acid) the urine was found to contain as salicylurates 60 % and as salicylic acid and salicylicglucuronides jointly 30 % of the

amount of salicyl administered (Schachter 1957). Quik (1932) reported the excretion of another drug commonly used in rheumatic diseases, i.e. para-aminosalicylic acid, as glucuronides, whereas for instance Muenzen, Cerecedo and Sherwin (1926) did not establish its presence. A great number of several corticosteroids and 17-ketosteroid metabolites are conjugated with glucuronic acid and, hence, after the administration of hydrocortisone 4 C^{14} about 60 % of the radioactivity excreted occurred in conjugation with glucuronic acid (Peterson, Wyngaarden, Guerra, Brodie and Bunim 1955).

II. OCCURRENCE OF COMPOUNDS OF GLUCURONIC ACID IN THE ORGANISM

Glucuronic acid as a structural component of connective tissue

General

The analysis of the structure of the connective tissue has recently been advanced considerably by morphological, histochemical and chemical research. Valuable contributions have also been received from physics (autoradiography, histospectrophotometry, etc.). Connective tissue or mesenchymal tissue occurs very generously in the organism, varying in character and quantity from organ to organ. It is very scanty in the lungs and endocrinous glands with the exception of the capsule. It is slightly more abundant in the liver and the kidneys. In the heart and the muscles connective tissue forms an important part of the viscus and the same also applies to the intestinal tract and the skin. The bones, joints and tendons, on the other hand, consist almost exclusively of connective tissue and it must be remembered that connective tissue is continuous throughout the body (Robb-Smith 1954).

The morphologically most essential components of the mesenchyme are the special cells of the tissue, special protein fibres and mucoid ground substance, i.e. intercellular substance. The composition of especially the last-mentioned part of connective tissue varies in the different organs, considerably influencing the properties of their mesenchymal tissue. Mucopolysaccharides, which are polymers of carbohydrates or their derivatives, are the most important constituents of the intercellular substance. These compounds seem to occur closely associated with protein or peptide residue and possibly in the form of salts (Artz and Osman 1951). One of the most common constituents of these mucopolysaccharides is glucuronic acid and it is in this form that the compound chiefly occurs in the organism. Only small quantities of other compounds of glucuronic acid are found.

Hyaluronic acid and its salts

Hyaluronic acid occurs in the organism in places where the tissue function is to bind water, and it also serves as a lubricant and shock-absorber in joints (Meyer 1954).

Hyaluronic acid was first isolated from the vitreous humour of the eye of cattle (Meyer and Palmer 1934) and later from the aqueous humour (Meyer and Palmer 1936) and cornea (Meyer and Chaffee 1940). The compound has also been isolated from human (Meyer and Palmer 1936) and pig (Follett 1948) umbilical cord, from human (in myxedema patients) (Watson and Pearce 1947), pig (Meyer and Chaffee 1941) and rat skin and the sexual skin of the monkey (in the estrogen phase) (Chain and Duthie 1940), from the synovial fluid of human and cat joints (Meyer, Smyth and Dawson 1938), from human synovioma and the liver metastases of a tumour (Meyer 1947) and probably from the nucleus pulposus of the intervertebral disc (Meyer 1947), from exudates (Campani 1942), from the viscous pleural fluid caused by a malignant tumour of the pleura and peritoneum (Meyer and Chaffee 1940), from viscous Rous and Fuyinami tumours in chickens (Pirie 1942) and also from fowl leucoses due to viruses (Kabat 1939) and from an endothelioma (Meyer and Chaffee 1940). Hyaluronic acid has also been found in bacteria, e.g. in the streptococci of the A and C group (Kendall, Heidelberger and Dawson 1937, Seastone 1939) and in Aerobacter aerogenes (Warren 1950).

Hyaluronic acid occurs either free or in the form of a salt-like protein complex from which it is separated with difficulty. In combination with protein this mucopolysaccharide forms a mucin "clot" with pH up to 4 (Artz and Osman 1951). Physiochemically bound hyaluronic acid has a protein content of c. 30 % (Kulonen 1951).

Hyaluronic acid is structurally a polymer of N-acetylhyalobiuronic-acid-disaccharide. The components of this disaccharide are acetyl-glucosamine and glucuronic acid (Meyer and Fellig 1950, Weissman and Meyer 1952, Kaye and Stacey 1950, 1951, Weissman, Rapport, Linker and Meyer 1953, Weissman, Meyer, Sampson and Linker 1954).

Mention must be made in connection with hyaluronic acid of hyaluronidase, an enzyme, which is capable of depolymerising hyaluronates right up to the disaccharide stage. The enzyme is found in organs (most used are the testis preparations) and in bacteria. Closely associated with the influence of hyaluronidase is the so-called "spreading reaction" in which hyaluronidase causes a manifest increase in the infection present e.g. in the tissue or culture (Hoffman and Duran-Reynals 1930, Duran-Reynals 1933).

The viscosity of normal synovial fluid of (human) knee joints is greater than the levels found in pathological conditions (Ragan 1946). The hyaluronic acid concentration in the knee joint fluids of cases of rheumatoid arthritis was the same as with normal patients (Ragan and Meyer 1949) or perhaps increased (Schürch, Viollier and Süllman 1950). As the volume of synovial fluid was greater in rheumatic than in normal cases, the total quantity was also greater (Ragan and Meyer 1949). The same workers found from viscosity determinations that the amount of incompletely polymerised hyaluronic acids increased in arthritic patients. The water-binding capacity was found to differ in rheumatic and traumatic effusions (Kulonen 1951).

Chondroitin sulphates and chondroitin

The chondroitin sulphuric acid compounds of the interstitial substance of connective tissue contain glucuronic acid as uronic acid. These compounds are: chondroitin sulphate A, B and C and chondroitin (few sulphate groups) (Meyer 1954).

Chondroitin sulphate A is the most important mucopolysaccharide component of hyaline cartilage and it is generally prepared of tracheal or nasal cartilages (Bray, Gregory and Stacey 1944, Partridge 1948, Meyer, Odier and Siegrist 1948, Einbinder and Schubert 1951, Mathews and Dorfman 1953). The compound has also been prepared from a human cystic chondrosarcoma (Meyer 1954) and from the cornea (Meyer, Linker, Davidson and Weissman 1953). Chondroitin sulphate is considered to be present in the cartilage in the form of protein salt (Mörner 1889, Meyer and Smyth 1937). Chondroitin sulphate A is a polymer from a compound containing equimolar quantities of N-acetylchondrosamine, glucuronic acid, and sulphate (Meyer 1954).

Besides hyaluronate, chondroitin sulphate B has been isolated from pig skin (Meyer and Chaffee 1941). It has also been isolated from tendon, cardiac valves and aorta (Meyer and Rapport 1951) and possibly from the skin of a normal and a myxedematous person (Watson and Pearce 1949). Equimolar quantities of uronic acid, hexosamine, acetyl, and sulphate have been established in the analysis of mucopolysaccharide (Meyer 1954).

Chondroitin sulphate C has been isolated from umbilical chord, tendon, cardiac valves and aorta (Meyer 1954). The compound has a similar composition to chondroitin sulphate A but differs from it in solubility, precipitation in certain substances and optical rotation. The amino sugar of the polysaccharide is chondrosamine; apart from this, the information on it is not altogether accurate. Malmgren and Sylvén in 1952 found the sulphate fraction of the nucleus pulposus to contain chondrosamine.

Chondroitin has been isolated from animal cornea (Davidson, Meyer 1954) and the compound is possibly the precursor of the chondroitin sulphates.

Meyer mentioned in 1944 the inability of connective tissue in scurvy to produce chondroitin sulphuric acid.

In chondromalacia the patella contains less chondroitin sulphuric acid than in normal cases (Hirsch 1944).

Goldberg (1944) regarded the thinning of the articular cartilage which occurs as an early symptom of rheumatoid arthritis as possibly associated with the chondroitin sulphuric acid metabolism.

Heparin

Fuchs (1930) found in the adventitia of aorta considerable quantities of a substance which prevented the clotting of blood. Charles and Scott (1931) found a large amount of heparin in bovine liver (more in the autolysed than in the fresh organ) and observed further considerable amounts in the muscle and the lungs, much less in the heart, thymus, spleen and blood.

Heparin is structurally built up of glucuronic acid, d-glucosamine and the sulphate group (Jorpes and Bergström 1936, 1939, Jorpes 1936, Wolfrom and Rice 1943, 1946).

The literature on heparin is voluminous, especially on the heparin content of the granules of mast cells; the cells have also been called heparinocytes (Jorpes 1946). The cell granules usually stain either metachromatically (Holmgren and Wilander 1937, Jorpes, Holmgren and Wilander 1937) or give a positive PAS reaction (Periodic-acid-Schiff-reagent) (Jorpes, Werner and Åberg 1948). Mast cells are generally localised in the walls of the blood vessels and they are fairly numerous in the ground substance of connective tissue.

Mucoitinsulphuric acid

Neutral and acid mucopolysaccharide has been isolated from pig stomach. The composition of the latter, mucoitin sulphuric acid, is d-glucuronic acid, acetyl glucosamine and ester sulphate (Meyer, Smyth and Palmer 1937, Wolfrom and Rice 1947).

Connective tissue mucopolysaccharide variations in collagen diseases

Acid mucopolysaccharides or metachromatic substances were produced profusely in the subacute stages of serous inflammatory lesions in rheumatic fever, rheumatoid arthritis and disseminated lupus erythematosus (Altshuler and Angevine 1951). The accumulation of mucopolysaccharides can be regarded as the result of the increased activity of fibroblasts and the process has been called mucoid degeneration (Ehrich 1952). The increase in acid mucopolysaccharides was often accompanied by an increase in mast cells. It is

possible that mast cells produce the amorphous ground substance of connective tissue and also acid mucopolysaccharides (Asboe-Hansen 1950, 1952).

Connective tissue displays in collagen diseases a so-called fibrinoid change, fibrinoid referring to some kind of homogeneous, eosinophilic, refractile, relatively acellular band-like structure which has some of the tinctorial properties of fibrin. It has been found in rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus and periarteritis nodosa (Altshuler and Angevine 1954).

Altshuler and Angevine (1949) expressed the opinion that fibrinoid is formed in connection with the co-precipitation of the acid mucopolysaccharides of the ground substance with a protein acting as a base. Their arguments were: (a) the temporal, spatial and configurational relationship of fibrinoid and metachromatic substances; (b) the positive polysaccharide reactions of fibrinoid with periodic acid leukofuchsin; (c) the increase in tissue pH and the liberation of alkaline substances in lesions generally associated with fibrinoid formation; (d) the failure to obtain consistently positive fibrin (phosphotungstic acid hematoxylin), lipoidal or Feulgen reactions in the fibrinoid material, and (e) the occurrence of fibrinoid or fibrinoid-like structures in areas where necrosis of muscle or of collagenic, reticular or elastic fibres cannot occur or where its occurrence is unlikely. This opinion, it was stated, could only be conclusively established by the isolation and chemical analyses of fibrinoid material from various anatomic sites.

Amyloidosis occurs relatively frequently in rheumatoid arthritis (Lövgren 1945, Teilum 1952, Teilum and Lindahl 1954, Laine, Vainio and Ritama 1955). Para-amyloidosis has been found in cases of disseminated lupus erythematosus (Teilum 1948, Aegether and Long 1949). Amyloid contains chiefly protein, but there is also c. 0.5—1.5 % of mucopolysaccharides and, on analysing the latter substances, uronic acid for instance has been found (Hass 1934); furthermore, in the paper electrophoretic determination of the amyloid substance only a smaller portion (about one-third) was found to contain uronic acid (Larsen 1957).

Clucuronic acid compounds occurring elsewhere in the organism

Small quantities of glucuronic acid are found in the blood conjugated with normal metabolism or toxic agents.

The plasma and serum glucuronic acid values reported in the literature vary in healthy persons from 0.4 to 4.5 mg per 100 ml (Deichman and Dierker 1946, Hollman and Wille 1952, Saltzman, Caraway and Beck 1954, Dowben 1956, Freeman, Kanabrocki and Inman 1956, Miettinen, Ryhänen and Salomaa 1957), from 4.0 to 9.2 mg per 100 ml in the blood (Rattish and Bullowa 1943, Fishman, Smith, Thompson, Bonner, Kasdon and Homburger 1951, Hollman and Wille 1952, Miettinen et al. 1957). In healthy persons serum glucuronide values range from 0.7 to 1.2 mg per 100 ml (Fishman and Green 1955).

Glucuronic acid and glucuronide levels have been observed in diabetes which are slightly elevated (Rattish and Bullowa 1943, Saltzman et al. 1954, Fishman and Green 1955) or within normal limits (Fishman et al. 1951).

In arthritic patients the mean glucuronic acid content of the blood was 5.8 for women, 5.5 mg per 100 ml for men (Fishman et al. 1951). In heart diseases the corresponding value was 5.1 mg per 100 ml.

Glucuronic acid occurs as a structural component of some immunopolysaccharides of the serum. The precipitin reaction of the artificial glucuronic acid antigen with types II, III and VIII of antipneumococci horse serum has been described (Goebel 1936). The antiserum of cellobiuronic acid-protein antigens conferred on mice a passive resistance to infection with virulent type II, III and VIII pneumococci (Goebel 1939).

When the glucuronic acid content of leucocytes was determined it was found to be low in chronic lymphatic leukemia, non-leukemic lymphocytosis, atypical and typical subacute myeloid leukemia. A very low glucuronic acid value was often established in chronic myeloid leukemia (Follette, Valentine, Hardin and Lawrence 1954).

Purulent cells were found to contain 6.5—8.2 mg per 100 ml of glucuronic acid. The amount of glucuronic acid present in spinal fluid was 0.5—2.6 and in pleural fluid 7.0—18 mg per 100 ml (Rattish and Bullowa 1943).

Bilirubin has been shown to be secreted in human bile as ester diglucuronide. Pigment II, the direct-reacting pigment of human bile in the diazo reaction of v.d. Berg forms azopigment B while bilirubin forms azopigment A. B has been found to be the ester glucuronide of pigment A. Another pigment reacting directly in azo reaction was pigment I which is perhaps an ester monoglucuronide of bilirubin (Billing 1957). Pigment I and II were found extensively, in excess of bilirubin even, in the (autopsy) bile of a patient with obstructive jaundice (Cole, Lathe and Billing 1954).

III. EXRETION OF GLUCURONIC ACID AND GLUCURONIDES IN THE URINE

Urinary glucuronic acid and glucuronides under normal conditions

Glucuronic acid in the urine of healthy individuals has long been investigated by several workers. The results they came to vary considerably, 65—1,325 mg per day (24 hours) depending on the method used. Low values were observed by Sauer 1930, Deichman 1943, Brox 1953, etc. Clearly higher values up to 600 mg and a little over were established e.g. by Maughan, Evelyn and Browne 1937, Südhof 1952, Hollman and Wille 1952, Kerby 1954, Dowben 1956, Freeman, Kanabrocki and Inman 1956, Miettinen, Ryhänen and Salomaa 1957. The highest values were found in the studies made by Wagreich, Kamin and Harrow 1940, Wagreich, Abrams and Harrow 1940, Hanson, Hills and Williams 1944.

In 1954 Kerby found the excretion of glucuronic acid in normal cases to be slightly lower (345 mg$\pm$ 44) for women than for men (391 mg $\pm$ 56). The difference was not significant.

In children, glucuronic acid excretion varied from 99 to 361 mg/24 hrs. (Tseng, Elghammer and Ivy 1951), mean 231 mg.

The total amount of glucuronides in the urine was 324—463 mg/24 hrs. in normal men (Fishman and Green 1955).

Many hormones are excreted as conjugates of glucuronic acid and certain hormones are elicited as fractions dissolved in some substances. Fractions dissolving in butyl alcohol and acetone have been examined in women during the menstrual cycle. The butyl alcohol fraction (G.B.S.) and the acetone fraction (P.G. which contains mostly pregnanediol) show a clear rise during the luteal phase in comparison with the follicular phase (Bisset, Brooksbank and Haslewood 1948, Jayle, Grepy and Meslin 1949).

A considerable increase in the butyl alcohol and acetone fractions and the pernanediol-glucuronidates has been established during pregnancy in normal individuals (Venning 1937, Jayle, Grepy and Tiprez 1949).

Glucuronic acid and glucuronides of the urine in pathological conditions

Cammidge (1906) believed that the excretion of glycuronic acid is increased in pancreatitis.

Brox (1953) studied the excretion of glucuronic acid in the urine of diabetes patients. He divided his material into three groups as follows: (1) acidotic, precomatous and comatous cases involving the risk of death, in which the urinary glucuronic acid values were 2.4—7.8 g in 24 hours; (2) cases which showed residual glycosuria but where there was no risk to life although complications were present; in this group the amount of glucuronic acid in the urine was 0.6—1.5 g per 24 hours; (3) diabetics whose rehabilitation was ideal — the excretion was 0.3—0.6 g of glucuronic acid in 24 hours. Brox found that the excretion of glucuronic acid increased even in slightly more severe diabetes.

Sauer (1930) and Brox (1953) reported that the glucuronic acids of the urine generally decreased in liver diseases. Low values were registered in acute and chronic hepatitis, liver cirrhosis, cholangitis. These two investigators found very low values in cases of hepatic coma. Bonomo and Colombo, on the other hand, in 1956, established increased values in acute icteric hepatopathy and mechanical icterus compared with the 24-hour excretion of glucuronic acid in normal individuals. The difference was statistically significant. Patients with chronic hepatopathy showed no demonstrable difference in glucuronic

acid excretion from normal cases. Following the administration of borneol, no difference was observed in borneol excretion between liver diseases and other diseases (Ottenberg, Wagreich, Bernstein and Harrow 1943). Borgström stated in 1949 that in liver diseases the benzoylglucuronic acid synthesis in disturbed more easily than the hippuric acid synthesis. The increase in glucuronic acid excretion is of no value in diagnosing mild liver lesions.

Pigments I and II were found in the urine in obstructive jaundice. The former might perhaps be regarded as an ester monoglucuronide of bilirubin and the latter forms together with benzenediazonium-chloride pigment B which is considered to be the ester glucuronide of pigment A formed by bilirubin in azo-reaction (Cole, Lathe and Billing 1954, Billing 1957).

On administering hydrocortisone -4C^{14} intravenously in congenital non-hemolytic icterus the urinary excretion of steroid glucuronides was found to diminished (Peterson and Schmid 1957). The ability to form other glucuronides did not disappear completely in the cases of this syndrome although it decreased in regard to other compounds apart from bilirubin (Axelrod, Schmid and Hammaker, in press).

In 1952 Geller observed low glucuronic acid excretion in a case of schizophrenia; it rose very sharply during insulin therapy.

In the disturbed conditions of the hormones regulating the menstrual cycle changes have been observed in the butyl alcohol (G.B.S.) and acetone soluble (P.G.) glucuronidate fractions. In hyperfolliculism, for instance, the G.B.S. and P.G. fractions showed a distinct rise in the follicular phase; and low G.B.S. and P.G. fraction values were found in the luteal phase in cases of hypoluteinism (Jayle, Vallin and Grepy 1950).

Südhof, in 1952, determined the glucuronic acid values of the urine of 17 patients with rheumatic diseases. He found lowered values in some chronic polyarthritis patients. In others the amounts were within normal limits. In a patient whit spondylarthritis ankylopoietica the glucuronic acid content of the urine was at the upper limit of normal, and one erythema nodosum patient had low values. After the intravenous administration of glucuronic acid lactone the excretion of glucuronic acid fell below normal in 3 cases of rheumatoid arthritis, 1 case of Bechterew's disease and 1 case of erythema nodosum.

Brummer, Savola and Leikkola, 1957, found high glucuronic acid and glucuronide values in a patient with spondylarthritis ankylopoietica.

No distinct difference was observed in the urinary glucuronic acid values of children with in active rheumatic fever compared with a control material. The few active cases probably showed some increase, but in these cases there were concomitant diseases (Tseng, Elghammer and Ivy 1950). Diminished glucuronide excretion was observed in 2 cases of rheumatic fever after the administration of borneol (Ottenberg, Wagreich, Bernstein and Harrow 1943).

IV. THE PRESENT INVESTIGATION

The object of the investigation and the problems

Glucuronic acid has been established as one of the structural components in hyaluronic acid, chondroitin sulphates, mucoitin-sulphuric acid, and heparin of the mucopolysaccharides in the interstitial substance of connective tissue. Variations have been observed in the amounts of mucopolysaccharides in rheumatic tissue, for instance in rheumatoid arthritis, rheumatic fever and disseminated lupus erythematosus in connection with degenerative changes. Such changes are mucoid degeneration, fibrinoid change, amyloidosis and para-amyloidosis (Altschuler and Angevine 1949, 1951, Teilum 1948, Klemperer 1950). As great changes occur in rheumatic diseases in both osseous and cartilaginous tissue as well as elsewhere in the connective tissue in which by far the greatest part of glucuronic acid occurs in the organism, it is of interest to find out whether the glucuronides of the urine show changes in rheumatic diseases. To this end, the investigation was concerned with examining the following problems:

(1) Are there differences in urinary glucuronides between rheumatoid arthritis and normal cases, and does the activeness and degree of severity of the disease have any effect on the excretion of glucuronides?

(2) Are the urinary glucuronide values affected by ACTH, cortisone and gold therapies in cases of rheumatoid arthritis?

(3) What is the situation regarding urinary glucuronides in other rheumatic diseases or in diseases affecting the joints?

Material

The series consisted of 202 cases (121 women, 81 men) of rheumatic, collagen and joint diseases. The mean age of the patients was 44.0 years. The patients had been hospitalised in the autumn of 1956 and spring of 1957 at the Medical Clinic of Turku University and the Hospital of the Rheumatism Foundation at Heinola. 73 healthy persons, mean age 32.4 years, from the personnel of the same hospitals served as controls during the same period. The controls consisted of 35 women and 38 men. Both materials were on a similar diet. For 4—5 days before the investigation and during it no medication was given. Because of the criteria decided on, several interesting cases in the active phase of the disease had to be discarded. On account of the glucuronide variations occurring in fertile women during the menstrual cycle the samples were taken 1 week after the termination of menstruation.

24-hour urinary glucuronide determinations were made in a total of 275 cases, in addition to which the glucuronic acid value was determined in 12 cases. 42 total protein, 38 serum paper electrophoretic, 36 serum mucoprotein and 22 bromsulphalein determinations were made parallelly with the urinary glucuronide determinations in cases of rheumatoid arthritis.

Methods

(1) The analyses employed for the determination of glucuronic acid.

Review of methods available: Decarboxylation methods: The principle of these methods is that by boiling glucuronic acid with hydrochloric acid (mostly 12 %) carbon dioxide is produced. This is measured volumetrically, gravimetrically or by titration (Mann and Tollens 1896, Lefevre and Tollens 1907, Dickson, Otterson and Link 1930, and Burkhart, Baur and Link 1934). The drawback of the method is that some carbohydrates form carbon dioxide in the process. Furfural is produced in connection with the decarboxylation reaction of glucuronic acid and its precipitation with phloroglusinol gives furfural phloroglucide. The substance evolved should be in a certain ratio to the quantity of glucuronic acid in the analysis (1: 3) (Gunther, de Chalmot and Tollens 1892, Lefevre and Tollens 1907, Tollens 1909, Ehrlich and Schubert 1929). The amount of furfural is not, however, quantitative but varies with the reaction conditions (Artz and Osman 1951).

Other methods involve colour reactions. When glucuronic acid compounds are in question, hydrolysis is performed by means of acids or β-glucuronidase and then the liberated glucuronic acid is determined.

By boiling Bial's reagent (orcinol, hydrochloric acid) and ferric chloride for a couple of minutes a greenish reaction is produced for glucuronic acid (Bial 1902). Scheff (1927) modified this method by using butanol as well. The method was modified further by using copper and acetic solution and was developed into a quantitative method (Jarrige 1950) employed e.g. for the determination of pregnanediol glucuronosidates in the urine (Jarrige 1950).

α-naphthol with sulphuric acid gives an emerald colour to glucuronic acid (Goldschmiedt 1910) and β-naphthol gives it a crimson red colour (Thomas 1925).

Some authors use the same methods as for sugar determinations, adapted for glucuronic acid. Quick (1924) extracted glucuronide with ether and performed the hydrolysis with hydrochloric acid, measuring the liberated glucuronic acid with either Benedict's reagent (sodium carbonate, sodium citrate, potassium cyanide, copper sulphate and

potassiumferrocyanide) or according to the method of Folin-Wu (copper tartrate in sodium carbonate solution, phosphomolybdic acid) (Quick 1926, Miller and Conner 1933, Miller, Brazda and Elliot 1933). According to Bertrand's method (copper oxide reduced from Fehling's solution reduces the ferrosulphate further and the iron salt thus formed is titrated with permanganate), glucuronic acid and glucurone reduce the copper in Fehling's solution slightly less than glucose. The copper equivalents of glucuronic acid have been determined (Goebel and Babers 1933, Kertesz 1935). Fishman (1939) determined the glucuronic acid evolved in splitting β-glucuronidase by means of the method of Miller and van Slyke (1936) (ferrocyanide and cerium sulphate, with Satopalin C as the indicator) (Levvy 1946).

In 1947 Dische observed that glucuronic acid or polyglucuronides and mannose in concentrated sulphuric acid, when allowed to react with thioglycolic acid, gave a deep pink colour which could be determined photometrically.

The above methods have been relatively little used for the determination of glucuronic acid and its compounds in urine. The most commonly employed methods are the carbazole and naphthorecorcinol methods.

In the colour reaction developed by Dische (1927, 1947) concentrated sulphuric acid is added first and then the alcohol solution of carbazole, where upon the colours is examined with a photometer according to Egami's method (1942). The reaction should be very specific to hexuronic acids and the method or its modifications have been used e.g. for the determination of glucuronic acid in the urine (Kerby 1954, Dowben 1956).

Tollens (1908) heated urine diluted with water to which an alcohol solution of naphthoresorcinol and hydrochloric acid had been added, shook the mixture with ether and observed that glucuronic acid gave a violet-blue colour. In 1909 Tollens introduced this reaction as a quantitative method. In the modifications of Tollens' reaction the acid hydrolysis is usually performed with hydrochloric acid or sulphuric acid, but several extraction agents are used and there are other additional features. The ether of the extraction medium was employed e.g. by Tollens 1909, Maughan, Evelyn and Browne 1937, Florkin and Crismer 1939, Kapp 1940, Mozolowski 1940, Wagreich Kamin and Harrow 1940, Deichman 1943, Ratish and Bullowa 1943,

Deichman and Dierker 1946. Jarrige (1947, 1950) used acetic acid, Hanson, Mills and Williams 1944 used amyl alcohol, De Frates and Boyd 1943, Miettinen, Ryhänen and Salomaa 1957 butyl acetate, and Fishman, Smith, Thompson, Bonner, Kasdon and Homburger 1951 and Fisman and Green 1955 employed toluene. All those methods have been used to determine the glucuronic acid and glucuronides in the blood, urine, other fluids of the body or in tissue "slices" or homogenates. In the determination of the glucuronides of certain hormones the first step must be extraction e.g. with butanol or acetone; then comes the glucuronic acid determination proper which involves still another extraction (e.g. with ether) (Bisset, Brooksbank and Haslewood 1948, Jayle, Grepy and Meslin 1949).

The method employed in the present investigation:

In the determination of total glucuronic acid co-incidental, disturbing compounds possessing an aldehyde group may be present. Hence it seemed that a more reliable method would be one in which total glucuronides could be determined and free glucuronic acid and the disturbing substances thus eliminated. For this reason it was decided to choose the glucuronide determination method reported by Fishman and Green (1955). In 1957 Brummer, Savola and Leikkola claimed that glucuronic acid values varied more than the corresponding glucuronide values. 32 glucuronic acid determinations were made in the present study parallelly with the glucuronide determinations. The variations were nearly identical in the two groups.

The principle in the method of Fishman and Green (1955) is as follows: Free glucuronic acid is eliminated from the mixture of glucuronic acid and glucuronides by oxidizing its free aldehyde group with hypoiodite at ph 10.1. Saccharic acid, which does not react with naphthoresorcinol, is then formed. Oxidation does not affect the glucuronides as aglycones protect the aldehyde group at carbon atom C_1. After this, the strong acids of the naphthoresorcinol test liberate the glucuronic acid from its conjugate and the free glucuronic acid reacts with naphthoresorcinol forming a violet compound which is determined photo-colorimetrically. In the determination of unconjugated acid the difference between the glucuronic acid values obtained before and after the oxidation procedure must be calculated. The determination is valid only when no disturbing quantities of hexose are present.

Reagents:

Toluene (Merck)
Ethyl alcohol, 95 per cent.
The buffer, pH 10.1. 8.4 g of sodium bicarbonate (Merck) and 36.0 g of sodium carbonate (anhydrous Merck) are dissolved in a litre of distilled water.
0.1N. iodine in potassium iodide solution.
Sodium bisulphite solution (1.0 N.), 10.4 g of $NaHSO_3$ are dissolved in 100 ml of distilled water. A fresh solution must be prepared every other week.
6N. sulphuric acid (1 volume 18N. sulphuric acid and 2 volumes of water).
18N. sulphuric acid (1 volume of concentrated sulphuric acid (Merck) is poured into 1 volume of distilled water and cooled to room temperature before use.
Aqueous solution of 0.2% naphthoresorcinol (Merck), always a fresh solution. (The naphthoresorcinol is pulverised with a pestle in a mortar; add water and shake vigorously for at least 10 minutes, filter). A paraffin oil bath is heated to 100°C and transferred to a thermostat.
Procedure: 5 ml of the sample to be tested (the urine was diluted 1:20—40 in this study) is pipetted into a Erlenmeyer flask capacity 50 ml with 2.05 ml of carbonate buffer. 1.5 ml of iodine solution is added and shaken carefully. The flask is stoppered and left in the dark for 30 minutes. 0.15 ml of sodium bisulphite solution is then added and the bottle is agitated. 0.3 ml of 6N. sulphuric acid is pipetted into it. Any pigment left by the iodine can be removed with a drop of bisulphite solution. The excess CO_2 is removed from the solution by careful shaking.

In determining total glucuronic acid, iodine, bisulphite and sulphuric acid are pipetted straight into 5 ml of the sample to be examined (the same amounts and order as above). The mixture is ready for the following step of the analysis and no 30-minute interval is required.

4 ml of the mixture is pipetted into special boiling tubes with a ground glass mouth. To each of them is added 2 ml of 0.2 % naphthoresorcinol and 2 ml of 18N. sulphuric acid. The contents of the tubes are mixed well. The tubes, unsealed, are placed in a 100°C oil bath for 90 minutes. The surface of the oil should reach to c. 2 inches from the tops of the tubes. After cooling (in cold water), 5 ml of 95 % alcohol is added to each tube and they are shaken 30 times to dissolve the pigment. 8 ml of toluene is pipetted into them.

The tubes are shaken 100 times very vigorously. The toluene extract is transferred to colorimeter tubes.

The tubes are allowed to stand in the dark for 5 minutes to clarify, after which the measuring is made in a Beckman photometer at the wave length 565 mμ.

Calibration curve: 5 ml of solutions containing 8, 16, 24, 32, 40, 48, 56 and 64 γ of glucuronic acid respectively are pipetted into Edlenmeyer flasks. The flasks already contain previously pipetted carbonate buffer, iodine solution, bisulphite and sulphuric acid (final volume 9 ml). 4 ml of this mixture, in duplicated samples, is pipetted for the naphthoresorcinol reaction. Fig. 3 gives in the form of a straight line the optical densities (as photometer readings) corresponding to 4 ml glucuronic acid concentrations.

Calculation: The average of two readings (optical densities) corresponds in a standard calibration curve (Fig. 3) to the glucuronide concentration of glucuronic acid in 4 ml. The values of free glucuronic acid are determined by deducting the glucuronide values from total glucuronic acid values.

Glucuronide solution: γ glucuronide x $\frac{9}{4} = \gamma$ glucuronide in 5 ml of analysis.

Urine (dilution 1:40)

$$\gamma \frac{glucuronide}{1000} \times \frac{9}{4} \times \frac{40}{5} \times 100 = \textit{mg per 100 ml of urine.}$$

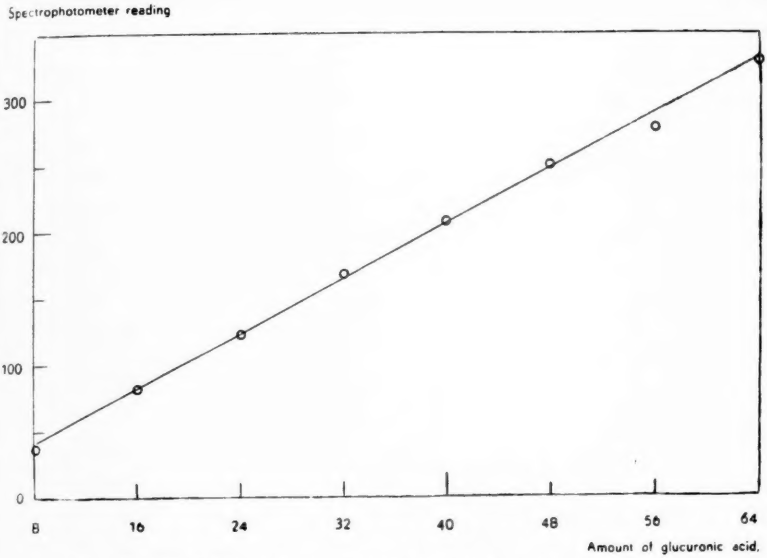


Fig. 3. — Calibration curve for glucuronic acid determinations.

Control

Fishman and Green (1955) emphasized the necessity of checking the standard curve (Fig. 3) several times. As, despite the careful observation of details, factors of detrimental effect may often occur in a biological test of this type, 2 samples (20—48 γ per sample) of glucuronic acid (Sigma Chemical Co. USA) were used throughout the investigation as duplicated samples.

Duplicated determinations were employed in the investigation. The necessity for this was emphasized by Fishman and Green (1955). The standard error of the duplicated determinations was estimated as follows (169 determinations):

$$S_n = \sqrt{\frac{\Sigma^2 \Delta}{2\,n}} = \sqrt{\frac{8568}{338}} = 5.03 \propto 5 \ mg$$

The error was thus around 1 %. The originators of the method found 1 % differences in duplicated determinations. They stressed the importance of careful heating, of testing the concentration of mineral acids and naphthoresorcinol, and of the pigment extraction technique. Apart from good laboratory technique, even heating was regarded as essential for accuracy. The result is closely dependent also on the observance of detail in preparing the naphthoresorcinol solution.

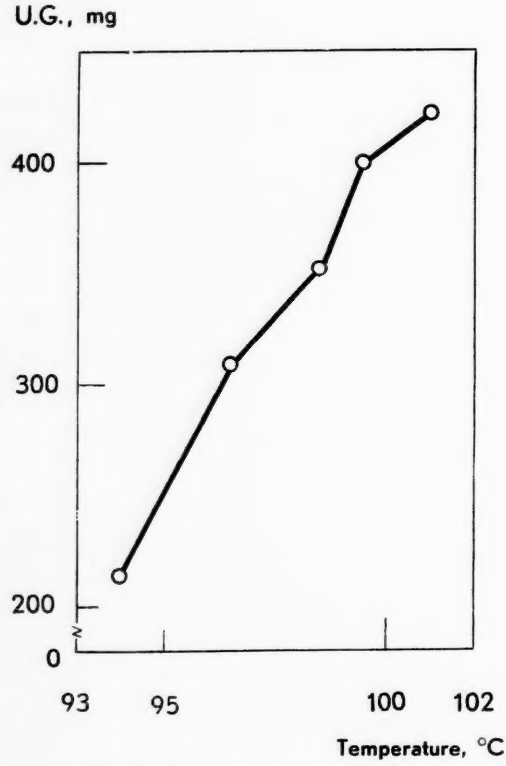


Fig. 4. — The effect of temperature on the results during acid hydrolysis of glucuronides.

Fig. 4 shows the effect of temperature on the result of the analysis. Continuous control of the temperature is a necessity.

Recovery test

To the original urine (symbol A in Table 1, volume 1,300 ml) was added phenolphthaleinmono-β-glucuronoside (Sigma Chemical Co. St. Louis USA), molecular weight 494.4. The compound thus corresponded to glucuronic acid (Mw 194.0) $= \frac{100 \times 194}{494.4}$ % = c. 39.2 %. To 100 ml of urine was added 15.0 mg of phenolphthaleinmono-β-glucuronoside which corresponds to 76.4 mg of glucuronic acid/1,300 ml of urine (sample C).

In sample B glucuronic acid was added to the urine correspondingly, viz. 38.2 mg per 1,300 ml of urine.

Table I

Recovery test

Amount of Phenolphtalein-mono-β-glucuronosidate (mg) Added to: No.	mg.	Total Theoretical Glucuronic Acid Value in 1300 ml Urine in mg	Recovered Amount in mg: Dublicated Determinations I	II	Mean	Error per Cent
A	—	516	—	—	—	—
B	97.5	554 (516 + 38 mg)	542	548	545	1.81
C	195	592 (516 + 76 mg)	591	597	594	0.34

Table 1 gives the means registered after the test i.e. 545 and 594 mg, amounts which correspond to the error percentages 1.18 and 0.34. The result was surprisingly good. Fishman and Green found a limit of c. 3 % satisfactory in the "recovery test".

Conservation of the urine: Certain micro-organisms are capable of destroying completely pregnanediol-type glucuronosidates in unconserved urine. Very effective in this respect are certain staphylococcus albus strains (Barber, Brooksbank and Haslewood 1948, Bisset, Brooksbank and Haslewood 1948). Venning (1939) stressed the importance of conservation. Hollman and Wille (1952) studied the

Table 2

Conserving of glucuronides in urine
(Determinations of urinary glucuronides in mg)

Urine — 4°C.		Test Days 13. 2.	15. 2.	18. 2.	22. 2.	25. 2.
With toluol	Case 1	522	517	519	468	436
,, ,,	Case 2	426	422	420	395	368
Without ,,	Case 1	522	478	436	402	—
	Case 2	426	391	357	339	—

ability of B.coli to use glucuronic acid as a nutrient. In the collection of urine, 10 ml of toluene was employed as a conserving agent and the urine was kept in a refrigerator at c. 4°C. The determinations were

generally done immediately after the collection of the urine or on one of the days immediately following. Table 2 shows the glucuronide changes in unconserved and conserved urine.

Diet: The patients and the controls ate during the investigations hospital food in which the variations in carbohydrate and protein content are relatively small. According to some workers (Hollman and Wille 1952), short-term nutritive variations (in carbohydrate, fat and protein content) do not affect the excretion of glucuronic acid. On a protein-rich diet a possible slight increase in the excretion of glucuronic acid was observed (Freeman, Kanabrocki and Inman 1956) (increase c. 5 %).

With very few exceptions, no albuminuria appeared in the patients. Furthermore, for several patients, the phenolsulphophthalein test was made and non-protein nitrogen determined; the values were mostly within normal limits. The urinary sediment was examined in all the cases.

(2) Other laboratory analyses

The *serum paper electrophoretic determination* was made according to the methods, sligthly modified, used by Grossmann and Hannig (1950) and Jenks, Jetton and Durrum (1955). The method uses a veronal buffer (pH 8.6) at room temperature. 0.1 ml of the serum is pipetted with the buffer on a wetted Whatman No. 1 paper strip. Tension is maintained at 250 V for 4 hours. The strips are dried in an incubator and allowed to stand for 16 hours in a solution containing bromphenol blue. They are then rinsed with 2 % acetic acid solution (finally Na-acetate solution containing 10 % acetic acid) and dried. The intensity of the colour is determined with a densitometer and the areas obtained measured with a planimeter.

Serum total proteins were determined by the Biuret method (Gleiss and Hinberg 1949, Aueswald and Bornschein 1949).

Serum mucoproteins were determined according to the method of Simkin, Bergman and Prinzmetal (1949). The other serum proteins were precipitated with 6 % perchloric acid. In this way the mucoproteins are not precipitated but remain in the solution which is filtered with Whatman (42). The mucoproteins are precipitated from the solution with 5 % phosphotungstic acid containing hydrochloric acid. The precipitate is dissolved in 0.2N. NaOH. Biuret reagent is added and the colour measured with a colorimeter (Coleman Jr.).

The *bromsulphalein test was* made according to Rosenthal and White (1925). 2 mg of bromsulphalein per kg body weight was injected into the patient intravenously. The samples were taken 5 and 30 minutes later and the colour intensity was determined.

The *erythrocyte sedimentation rate* was determined according to Westergren and the *hemoglobin count* according to Peters and v.Slyke (1933).

3. Statistical analysis

The mean, the standard deviation s and the standard error of the mean ε are given for a general description of the data. For the standard deviation, the classical formula has been applied:

$$s = \sqrt{\left[\Sigma x^2 - (\Sigma x)^2/n\right]/(n-1)}.$$

In the formula, x stands for the individual observations, n being the number of cases. The standard error of the mean ε was computed according to the formula

$$\varepsilon = s/\sqrt{n}.$$

In some cases, differences between successive observations for the same individual were calculated. The statistical treatment of these differences follows the above presentation, except that now x only represents a single observed difference.
In comparing two means, the standard error $\varepsilon\Delta$ of the difference of the means is obtained by the formula

$$\varepsilon\Delta = \sqrt{\varepsilon_1^2 + \varepsilon_2^2},$$

where the sub-indices refer to the two groups. The difference of the means divided by this standard error yields the test variable t, the statistical significance of which can be determined by means of the appropriate statistical tables (Fischer & Yates 1953). In connection with the tests of significance, the probability P is given: P indicates the chances that the difference is due to random fluctuations only. If these chances are sufficiently small, say $P < 0.05$, the difference is said to be statistically significant.

For testing the significande of the mean change of excretion from one period to another, this mean is divided by its standard error. Again, a *t*-value is obtained the significance of which can be determined by consulting statistical tables.

When comparing simultaneously more than two means, a more complicated technique known as analysis of variance was applied. This technique is well described in e.g. Fischer (1950).

The analysis of dependence between, say, excretion and some protein fraction was performed by means of correlation analysis. This analysis, the presupposition of which is linear dependency between the two variables, is also described in the excellent text-book of Fischer (1950). If an essential dependency is observed, the result is said to be statistically significant. Otherwise, the probability P that the numerical dependency is due to random causes only is larger than 0.05, $P > 0.05$. In some passages, correlation analysis is also called regression analysis.

In order to establish the variability or the error of the method S_M, a classical formula was applied based on duplicated determinations. According to this formula, the standard error of a single determination is estimated from

$$S_M = \sqrt{\Sigma\, d^2/2n},$$

where d stands for the difference of a pair of determinations, n being the number of duplications.

V. URINARY GLUCURONIDES IN HEALTHY PERSONS

General

Women. — Urinary glucuronides were determined on two successive days in 33 healthy women (plus in 2 cases on one day). The age range was 19 to 56, average 30.9 years. The mean of urinary glucuronides during 24 hours was 407 mg (standard error, (S.E.), 12 mg, Table 3). There were no statistically significant differences in the different age groups (under 24 years, mean 400.9 mg, S.E. 23. 9; 25—39 years 407.3 mg, S.E. 11.6; over 40 years 416.1 mg, S.E. 21.9).

Table 3

Urinary glucuronides (U.G.) in healthy persons, (mg)

Group	Number of Cases	Age of Cases, years Mean	Mean of U.G. mg	Standard Deviation of U.G.	Standard Error of Mean
Females . . .	33	30.9	407	58	10
Males	38	33.9	444	72	11
Total	71	32.5	427	39	12

Men. — The control series comprised 38 cases for all of which urinary glucuronides were determined on two successive days. The age range was 20 to 62, average 33.9 years. The mean urinary glucuronide quantity during 24 hours was 444 mg (S.E. 11, Table 3). No statistical differences were found between the different age groups (under 24 years, mean 464.9 mg, S.E. 21.9; 25—39 years, 429.6 mg, S.E. 18.7; over 40 years, 449.5 mg, S.E. 17.3).

The mean urinary glucuronide quantity in healthy men was higher than the corresponding value for women. The difference was statistically significant ($t = 2.40$, $P = 0.02$). The mean urinary glucuronide quantity of the entire material of healthy persons was 427 mg (S.E. 10).

Variations during the inter-menstrual period

The excretion of glucuronides between the menses was observed in 14 healthy, fertile women (Fig. 5). The glucuronide determinations were made as follows: (I) 1—2 days after menstruation, (II) about a week later, (III) 14—18 days from the beginning of menstruation (time of ovulation), (IV) a week after sample III and (V) 1—2 days before menstruation (Fig. 5). In 4 cases (Nos 12, 20, 30, 31) menstruation began unexpectedly early and determination V was not made. In case 9, on the other hand, menstruation was delayed by 10 days.

The lowest values before and after menstruation were: the mean of sample I 374 mg, of sample V 420 mg. The highest average value was obtained with sample III (ovulation period), 477 mg, but the difference between samples III and IV (mean 445 mg) was not statistically significant ($P = 0.1$). On the other hand, the variations between the means of samples I—II, II—III and IV—V were statistically significant ($P = 0.01$).

VI. URINARY GLUCURONIDES IN RHEUMATIC DISEASES

Rheumatoid arthritis

General, the effect of sex

The series comprised 53 men and 80 women suffering from rheumatoid arthritis. The mean age of the women was 49 years (range 25—75) and of the men 43.5 (range 20—64).

Women. — *A* 24-hour urinary glucuronide determination was made on two successive days in 77 cases and on a single day in 3 cases. In 77 cases the mean amount of urinary glucuronides was 356 mg (S.E. 7). Compared with the corresponding mean value, 407 mg. for healthy women the decrease in glucuronides was statistically significant (P = 0.001) (Table 4).

Table 4

Urinary glucuronides (U.G.)/24-hours in mg in rheumatoid arthritis

Group	Number of Cases	Mean of U.G. /24 h.	Standard Deviation	Standard Error	Comparison with the Corresponding Healthy Group	
					t	P.
Females	77	356	62	7	4.10	0.001
Males	53	398	48	7	3.49	0.001

Men. — Urinary glucuronides were determined in all the 53 cases on two successive days; the 24-hour mean value was 398 mg (S.E. 7). The corresponding value for healthy male controls was 444 mg. Hence,

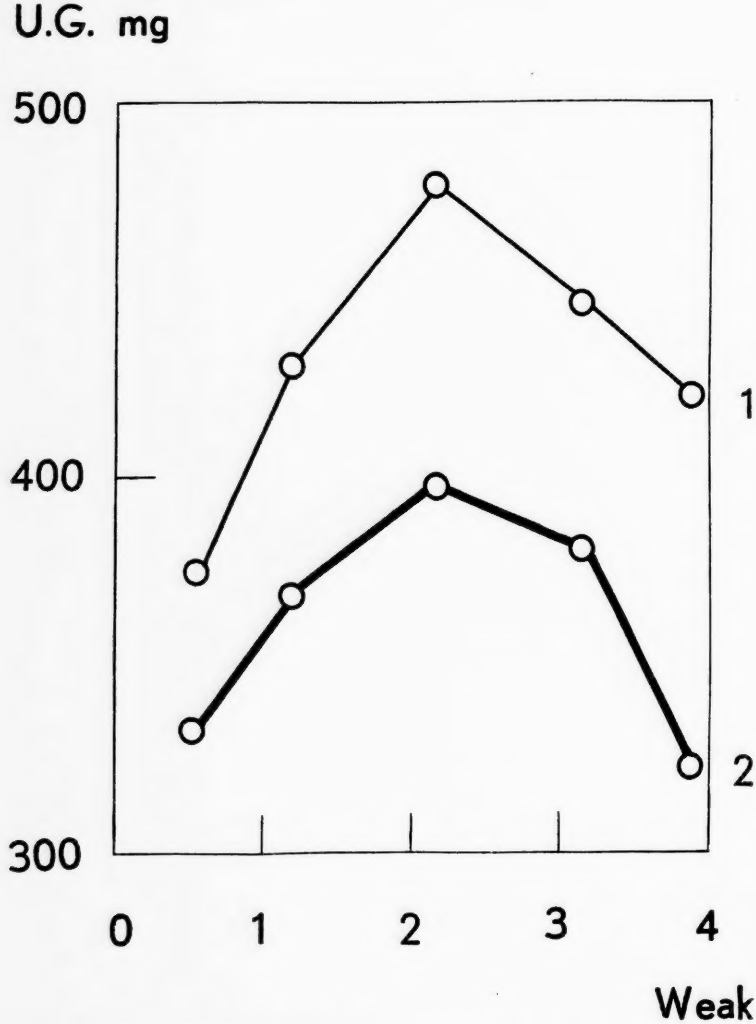


Fig. 5. — Changes of urinary glucuronides (U. G.) during the intervals between menstruations in female healthy and rheumatoid arthritis patients. Curve 1 healthy, curve 2 female rheumatic.

compared with the controls, urinary glucuronides decreased also in men suffering from rheumatoid arthritis; the difference was statistically significant ($P = 0.001$).

There were no statistically significant differences between the different age groups in either sex.

Variations during the inter-menstrual period

Urinary glucuronides were observed during the menstrual cycle in cases of rheumatoid arthritis in the corresponding periods as for healthy women (sample I, 1—2 days after menstruation; II about a week later; III 14—18 days from the beginning of menstruation; IV a week after III; and sample V 1—2 days before menstruation). It was impossible to withhold medication from the patients because of the severity of the disease and therefore not all the samples were taken from the same patient. Fig. 5 shows the intermenstrual variations. A similar variation of urinary glucuronides during the menstrual cycle to that seen in healthy women was observed in the rheumatoid arthritis cases. The values were lowest immendiately before and after menstruation and the highest values occurred during the luteal phase in samples III and IV. The differences are so clear that they must inevitably appear in fertile female patients. Consequently, the samples were taken about a week after menstruation. No significant differences were observed in urinary glucuronide values before and after the climacterium ($t = 0.78$).

Relation to the stage of the disease

The classification of rheumatoid arthritis cases in 4 different degrees of severity on the basis of the clinical and roentgenological picture, as approved by the American Rheumatism Association (Steinbrocker, Traeger and Batterman 1949), is as follows:

Stage I (Early): There may be osteoporosis roentgenologically but no destructive changes.

Stage II (Mild): (1) Osteoporosis is demonstrated roentgenologically; slight cartilage or sub-chondral bone destruction may occur. (2) There are no joint deformities although there may be some limitation in the motion in the joints. (3) Mild muscle atrophy. (4) Extra-articular lesions such as nodes and tenovaginites may be present.

Stage III (Severe): Cartilage and bone destruction may be demonstrated roentgenologically together with osteoporosis. (2) Joint deformities such as subluxation, ulnar deviation or hyperextension without fibrous or bony ankylosis. (3) Extensive muscle atrophy. (4) Extra-articular changes as in Stage II.

Stage IV (Terminal): (1) Fibrous and bony ankyloses. (2) Criteria of Stage III.

The distribution of the cases into the above groups is given in Fig. 6 and Table 5. In 53 male cases the urinary glucuronide means declined fairly evenly with the stage of the disease, though without significant differences. However, the material of group IV is too small for a comparison. The 77 female cases did not show a similar decrease in glucuronides except between stages III and IV where the difference was almost significant ($P = 0.05$).

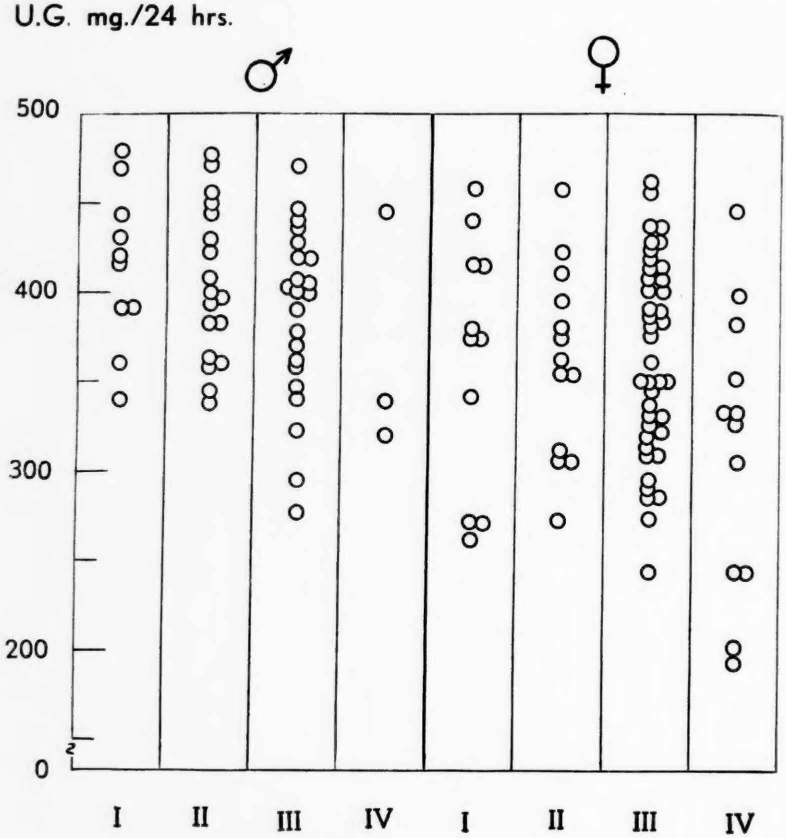


Fig. 6. — Distribution of urinary glucuronides by stage of the disease in arthritis rheumatoid material.

Table 5

Stage of rheumatoid arthritis and urinary glucuronides (U.G.) in mg

Stage	Male Cases No.	U.G. mg Mean	Female Cases No.	U.G. mg Mean
I	10	416	11	364
II	18	405	13	363
III	22	387	41	364
IV	3	369	12	314

No significant difference occurred on comparing groups I and II with groups III and IV with either men or women.

Relation to the patient's functional capacity

Steinbrocker, Traeger and Batterman (1949) distributed their rheumatoid arthritis cases into 4 classes by the patient's ability to perform normal functions:

Class I (Complete). Ability to carry on all usual ruties without handicaps.
Class II. (Adequate) for normal activities despite handicap of discomfort or limited motion at one or more joints.
Class III. (Limited) only to little or none of duties of usual occupation or self care.
Class IV. (Incapacitated), largely or wholly. Bedridden or confined to wheelchair; little or no self care.

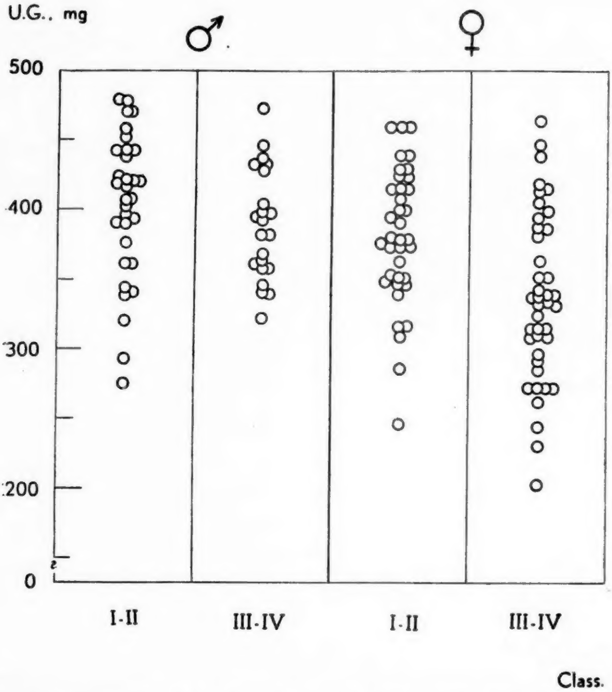


Fig. 7. — Distribution of urinary glucuronides by functional capacity of patients in rheumatoid arthritis cases.

In Fig. 7 and table 6 the urinary glucuronides in 77 female and 53 male patients are distributed into classes according to functional capacity. For the statistical comparison, classes I and II were taken

together, likewise classes III and IV. Amongst men, no difference seemed to exist between the groups ($t = 1.06$, $P = 0.1$). With women, the difference between the mean urinary glucuronide values of the groups was statistically significant ($t = 3.82$, $P = 0.001$).

Table 6

The functional capacity and urinary glucuronides (U.G.)

Class	Male Cases No.	U.G. mg Mean	Female Cases No.	U.G. mg Mean
I & II	31	403	36	383
III & IV	22	390	41	333

Relation to the duration of the disease

The average duration of the disease in the material as a whole was 6.3 years, 6.0 for men and 6.5 for women. No significant differences were observed either in men (means 405 and 391 mg) or in women (means 357 and 353 mg) between the urinary glucuronide values of cases in which the disease lasted less than and over 3 years.

Relation to the hemoglobin

A concomitant of rheumatoid arthritis is generally anemia which is correlated with the degree of severity of the disease and, as with secondary anemias usually, relatively resistant to therapy. For the total series the mean hemoglobin value was 12.1, 11.4 for women and 13.1 g per 100 ml for men. In the groups with hemoglobin values under and over 10 g per 100 ml for women there was a significant difference ($t = 4.92$, $P = 0.001$) in urinary glucuronide values. Nothing conclusive can be said of the difference in men as only 2 men gave a Hgb value of under 10 g per 100 ml.

Relation to fever

Fever is an indication of the activeness of rheumatoid arthritis and severe fever was in fact characteristic, often from the initial phase of the disease, of a group of these cases. Fig 8 and Table 7 show the

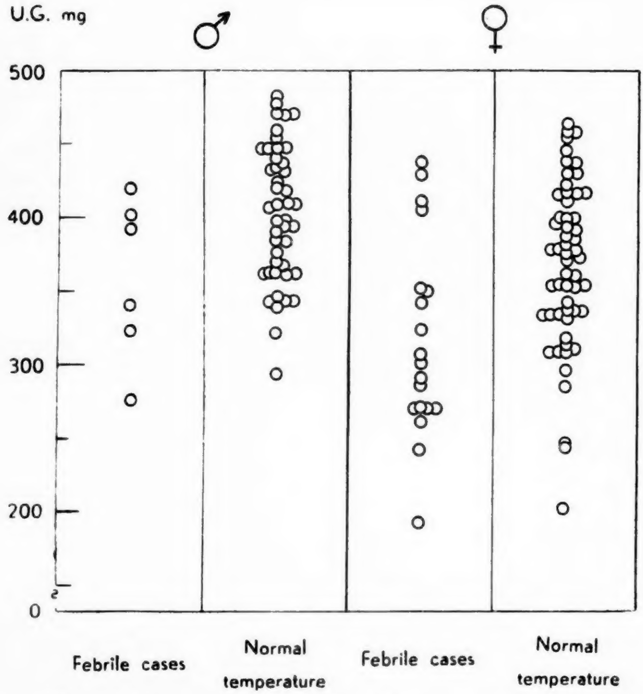


Fig. 8. — Effect of fever on urinary glucuronides in rheumatoid arthritis cases.

distribution of urinary glucuronide values in male and female patients according to fever. Among the men there were only 6 febrile patients and these did not show any significant difference in urinary glucuronide values from the other cases ($t = 1.83$, $P = 0.1$). A statistically significant difference was observed in female patients between the febrile cases and the nonfebrile cases ($t = 3.12$, $P = 0.01$).

Table 7

Correlation of fever with urinary glucuronides in rheumatoid arthritis

Group	Male Cases No.	U.G. mg Mean	Female Cases No.	U.G. mg Mean
Febrile cases	6	359	20	318
Nonfebrile cases	46	404	56	369

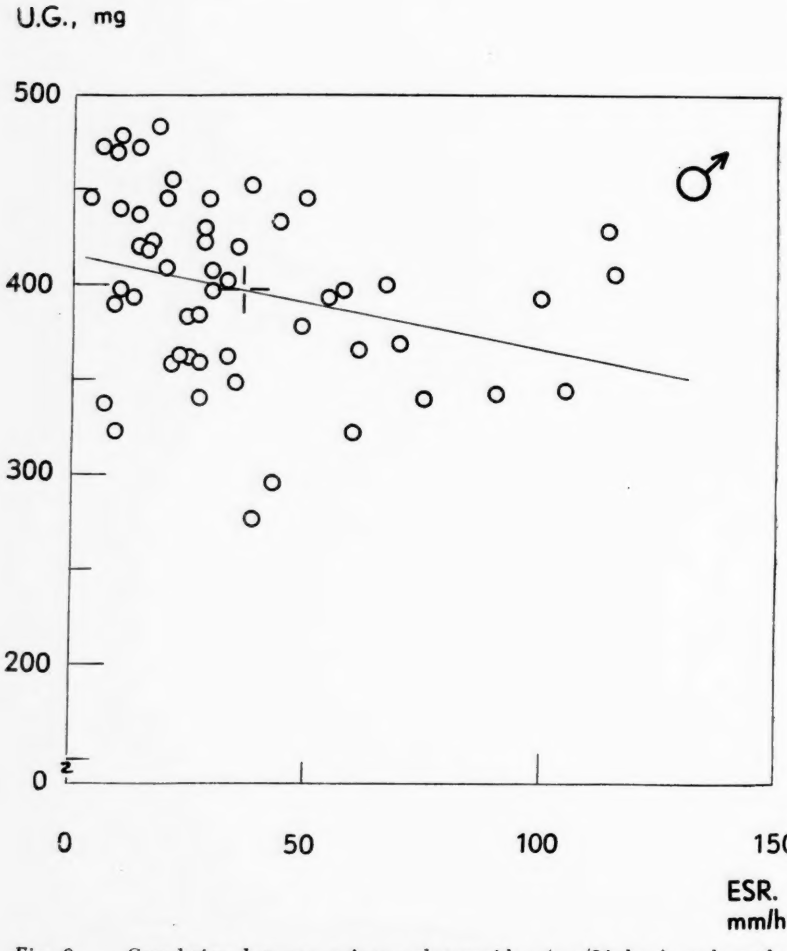


Fig. 9. — Correlation between urinary glucuronides (mg/24 hrs.) and erythrocyte sedisedimentation rate in female rheumatoid arthritis. The regression line corresponds to the equation: Urinary glucuronides = 403 — 0.98. ESR.

Relation to the erythrocyte sedimentation rate

The average erythrocyte sedimentation rate of 77 female patients with rheumatoid arthritis was 48 mm/hour; for 53 men it was 36 mm/hour. The correlation of urinary glucuronides with the sedimentation rate was studied separately for men and women by means of regression analyses (Figs. 9, 10). Statistically significant regressions

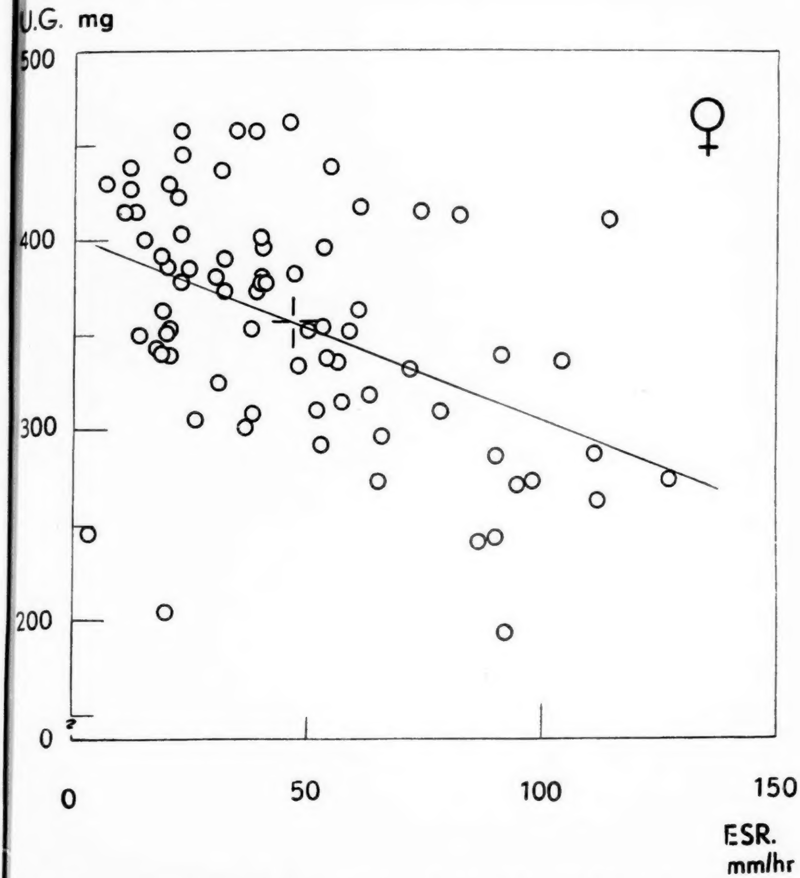


Fig. 10. — Correlation between urinary glucuronides (U. G.) and erythrocyte sedimentation rate in male rheumatoid arthritis. The regression line corresponds to the equation U. G. = 415 — 0.48 x ESR.

were established in both men and women but as the deviation was relatively great (Fig. 9) the correlation does not seem to be especially convincing biologically. Urinary glucuronide values seem to decline with the increase in the sedimentation rate and this would seem to suggest lowered glucuronide values in active rheumatoid arthritis cases.

Relation to the electrophoretic pattern of serum protein

Paper electrophoretic fractionation of serum proteins was performed on 39 patients with rheumatoid arthritis. Total proteins were

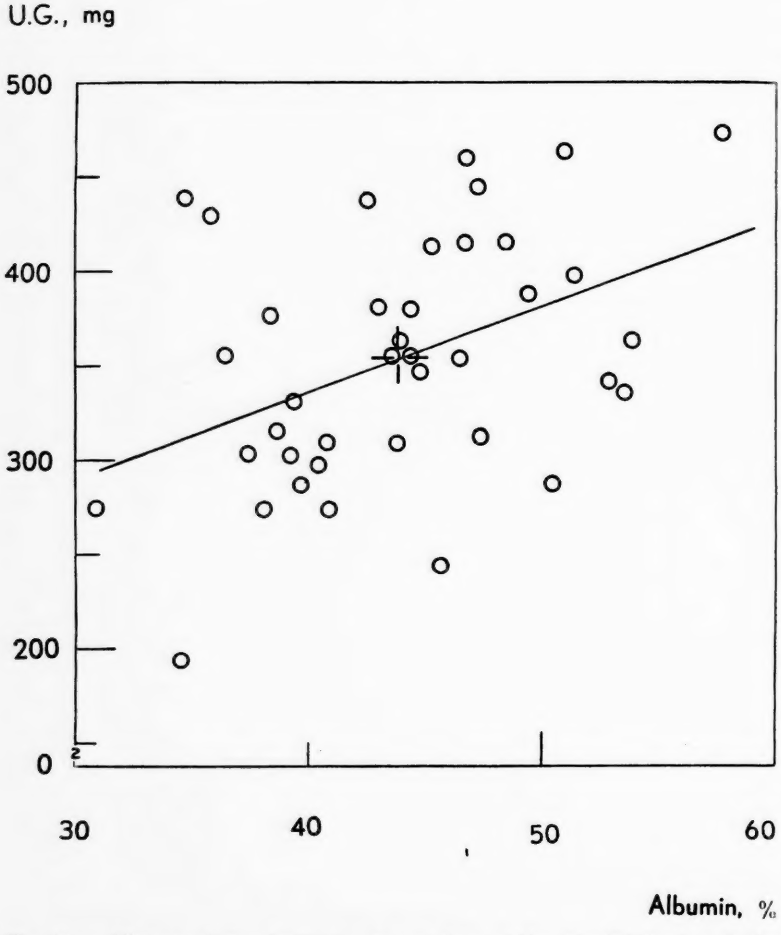


Fig. 11. — The correlation between urinary glucuronides (mg/24 hrs.) and the serum paperelectrophoretical albumin fraction (%) in rheumatoid arthritis. The regression line corresponds to the equation: U.G. = 158 + 4.45 x Albumin.

determined in these cases and also in 3 other patients and compared with the urinary glucuronide values.

There was no correlation between urinary glucuronide values and total proteins. Correlating the different fractions of serum proteins with urinary glucuronides revealed that the albumin fraction showed a statistically significant regression as regards the glucuronide values ($P = 0.01$). When the amount of albumin decreased the urinary glucuronide values also seemed to diminish. This is shown as a regression

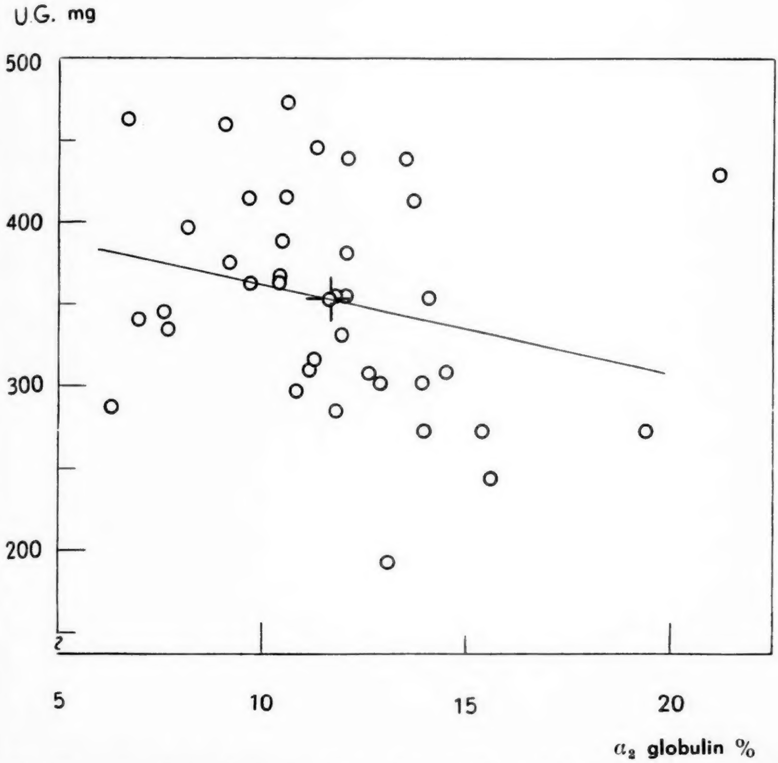


Fig. 12. — Correlation between urinary glucuronides and the serum paperelectrophoretical $\alpha_2$globulin fraction in rheumatoid arthritis. The regression line corresponds to the equation: U. G. = 416 — 5.54 $\alpha_2$globulin. The relation is not statistically significant.

line in Fig. 11. The ratio of α_2 globulin and the quantity of urinary glucuronides is similarly shown in Fig. 12. When the α_2 globulin fraction increased the urinary glucuronide values seemed to decline, but the regression was not significant.

There was no correlation between the α_1, β and γ globulin fractions and the urinary glucuronide values. The means of these protein fractions were 5.3 % for α_1, 13.2 % for β and 25.8 % for γ globulins.

The increase in the severity of the rheumatoid arthritis process was accompanied by an increase in all the globulin fractions, most markedly in the α_2 globulin, and correspondingly by a decrease in the albumin fraction. The decrease in serum albumin is regarded as a more sensitive indicator of activeness than the increase in globulins

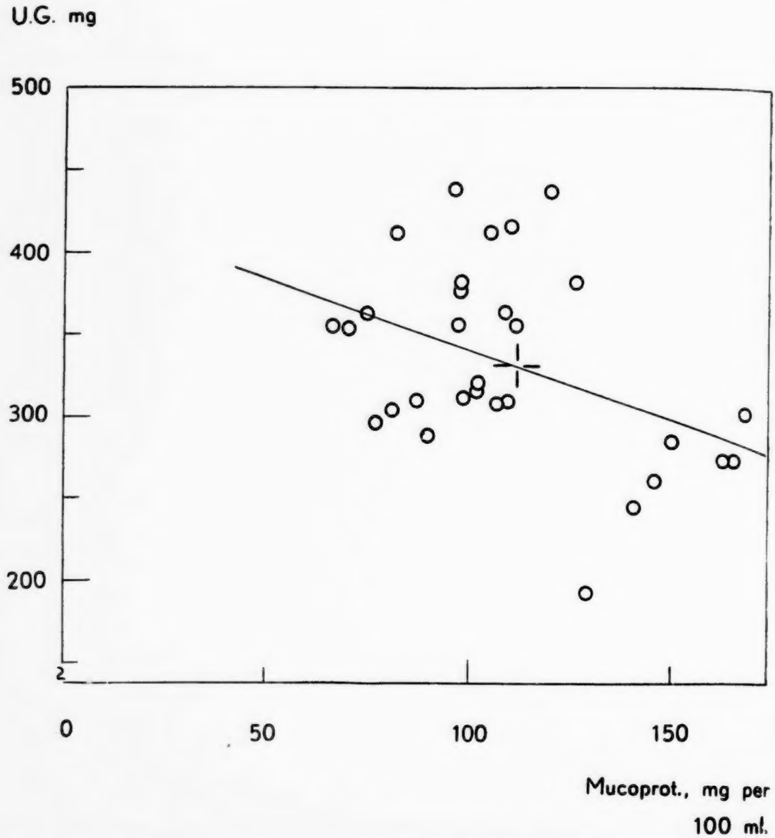


Fig. 13. — Correlation between urinary glucuronides and the serum mucoprotein fraction (mg %) in rheumatoid arthritis. The regression line corresponds to the equation U. G. = 427 — 0.86 Mucoprotein.

(Ropes, Perlmann, Kauffman and Bauer 1954, Kukowka 1955, Kalliomäki 1956, Stidworthy, Payne, Shetlar C and Shetlar M. 1957). The cases examined showed a decrease in the urinary glucuronide values with the decline of the serum albumin fraction and a similar tendency appeared when the α_2 globulin fraction increased. These observations point to the lowering of the urinary glucuronide values in the active stages of rheumatoid arthritis.

Relation to the serum mucoprotein content

Serum mucoprotein values were determined for 31 patients parallel with the urinary glucuronide determinations. The mean quantity of

mucoproteins was 112 mg per 100 ml. The regression line in Fig. 13 illustrates the comparison of serum mucuprotein and urinary glucuronide values. A statistically significant correlation was observed between these values (P = 0.05).

Mucoproteins have been found to increase in infectious diseases (e.g Winzler and Smyth 1948, and Winzler, Devor, Mehl, and Smyth 1952, and Greenspan 1954). Higher values were demonstrated earlier in inflammatory rheumatic diseases than in degenerative articular and spinal diseases. The mucoprotein fraction was found to rise more commonly in very active rheumatoid arthritis cases than in less active cases (Camp and Bopp 1955). Urinary clucuronide values seemed to decline when mucoprotein values increased and this observation supports the view that urinary glucuronide values seem to fall in active rheumatoid arthritis cases.

Relation to liver function

The bromsulphalein test was employed to measure liver lesion. It was performed on 24 cases of the material. 6 cases showed over 10 % retention half-an-hour after the injection of the dye. Increased bromsulphalein retention was demonstrated previously in 17 % of 100 cases of rheumatoid arthritis (Laine, Holopainen and Koskinen 1955). The regression curve in Fig. 14 illustrates the correlation between the bromsulphalein test and the urinary glucuronide values. The regression was not statistically significant. The quantity of urinary glucuronides seemed to diminish with the increase in the bromsulphalein values.

The liver is one of the organs in which glucuronide formation has been shown to occur with several substances. Impaired liver function should be seen in the formation of glucuronides. Indeed, the cases examined did show a tendency to falling glucuronide values. These cases seemed to involve a very slight reduction in liver function. In the bromsulphalein test the liver parenchyma is taken to secrete a great portion of the dye and the extrahepatic reticuloendothelial system to have a secondary role (Brauer and Possotti 1947, Lichtman 1953). The latter factor, however, must be taken into consideration.

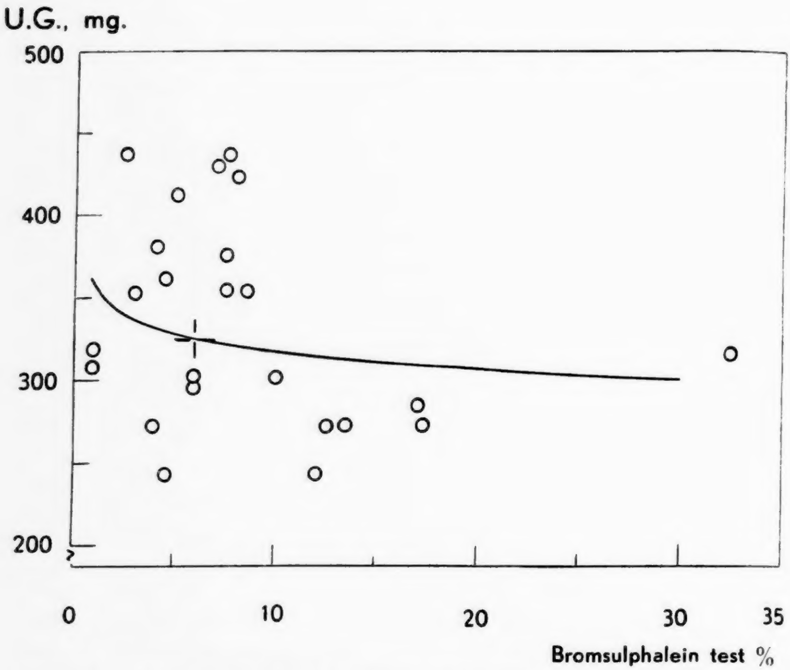


Fig. 14. — Correlation between urinary glucuronides and the bromsulphalein test (%) in rheumatoid arthritis. The regression curve corresponds to the equation: log. U. G. = 2.87 — 0.050 x log. Bromsulphalein (%) (Briggs'log.). The relation is not significant.

Gold therapy

Both the 77 female and the 52 male cases of rheumatoid arthritis were each distributed into 3 groups as follows: (I) Cases which were not given gold; (II) Patients who received less than 0.3 g of gold; (III) Cases given more than 0.3 g of gold, regarded as having received "prolonged" treatment. Fig. 15 and Table 8 show the distribution of the total material into 6 groups.

Table 8

Gold therapy and urinary glucuronides (U.G.) in rheumatoid arthritis

Croup	Male Cases No.	U.G. mg Mean	Female Cases No.	U.G. mg Mean
No treament	18	402	34	336
Gold < 0.3 g	19	399	30	377
"Prolonged" therapy	15	388	13	362

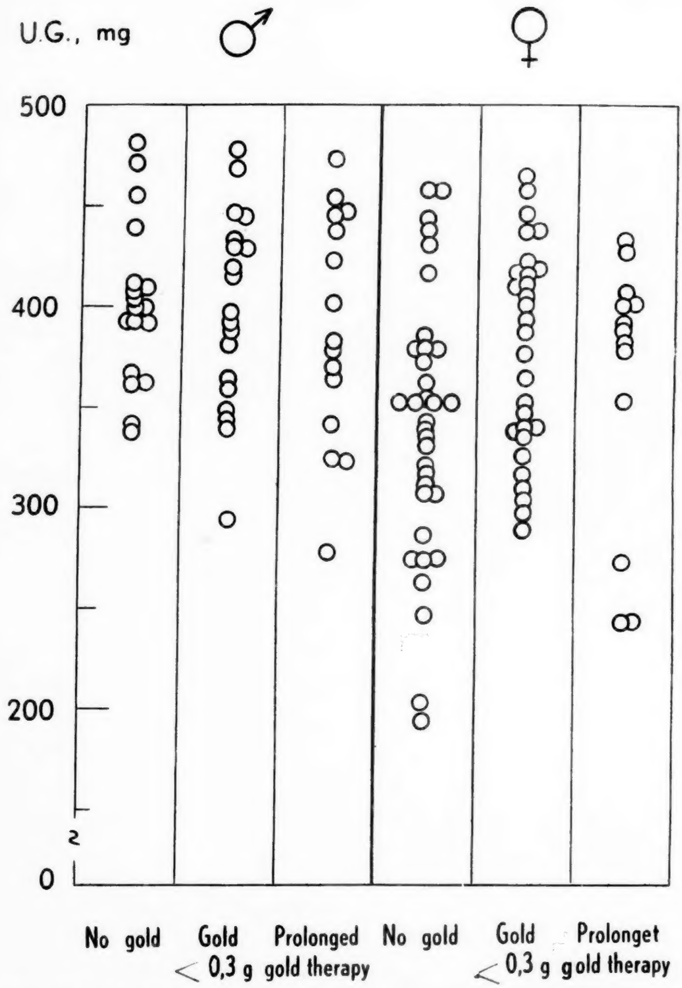


Fig. 15. — Distribution of urinary glucuronides by gold therapy in rheumatoid arthritis.

No significant differences were observed between the different male groups. The women showed perhaps an almost significant difference between groups I and II ($P = 0.05$); the cases given gold showed a tendency towards rising urinary glucuronide values. However, it is important to note the effect of the degree of severity of rheumatoid arthritis, activeness, etc.

It has been held that gold therapy might possibly affect the liver function test results (e.g. the bromsulphalein test; Laine, Holopainen

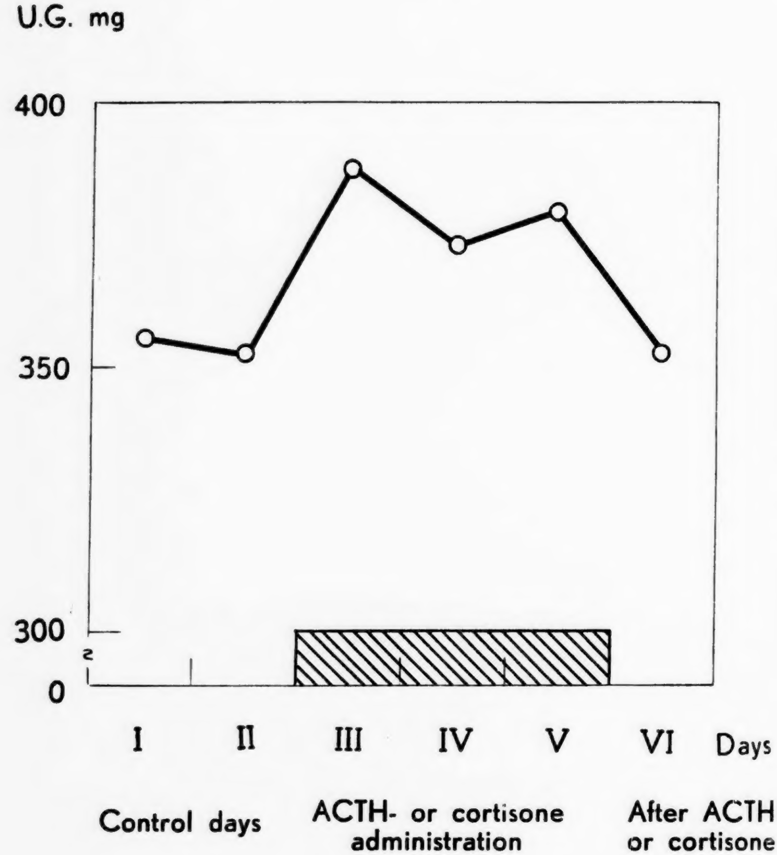


Fig. 16. — Effect of ACTH and cortisone administration on urinary glucuronides in rheumatoid arthritis.

and Koskinen 1955). Gold has been suspected of a hepatotoxic influence (Goodman and Gilman 1941, Hench 1947, Archer 1950 etc.). As the women showed a tendency to increase the quantity of urinary glucuronides and no appreciable differences were demonstrated in the men, no conclusion can be drawn concerning the effect of gold preparations in the doses generally used on glucuronide detoxication.

Administration of ACTH and cortisone

Thirty-three patiens with rheumatoid arthritis were given either ACTH (80 units of long-acting ACTH per day on 3 days) or corti-

sone (cortisone on 3 days: 1st day 300 mg, 2nd day 200 mg, 3rd day 100 mg). Urinary glucuronide determinations were made on two control days before the administration of the drugs, during their administration and one day after it. The function of the adrenal cortex was examined by performing Thorn's test (1948) on 22 patients of the series.

Fig. 16 shows the variations of urinary glucuronide quantities during the administration of ACTH and cortisone. The values showed an increase immediately after the administration of steroids was started and the difference from the control day was statistically significant ($t = 3.96$, $P = 0.001$), during the 3 days of administration of steroids no statistically significant differences were observed in the urinary glucuronide values although there were differences. A statistically significant difference was observed between the last day of steroid administration and the level on the following day ($t = 3.18$, $P = 0.01$). The quantity of glucuronides was found to be increased on the days of steroid administration compared with the preceding and following days.

Fig. 17 shows the relation of the urinary glucuronide variations observable during the administration of steroids and the fall in eosinophil cells demonstrated in Thorn's test. There seemed to be no correlation.

The administration of ACTH and cortisone thus seemed to increase the amount of urinary glucuronides at least on a few days. Animal tests also showed that cortisone increased the excretion of glucuronic acid in the urine (Cessi 1954). The present writer also noted in the series of rheumatoid arthritis the effect of long-term steroid therapy on urinary glucuronides. The series comprised 10 cases given cortisone regularly for over 6 months. 3 of them were men with a 24-hour mean urinary glucuronide value of 391 mg; the corresponding mean of the total male series was 398 mg. For the 7 female patients with rheumatoid arthritis the mean was 307 mg, the mean for the total female group bei 356 mg. The female mean of 307 mg was low, but the group was small, and it is consequently impossible to come to any definite conclusions regardig the effect of long-term cortisone therapy on 24-hour urinary glucuronide values.

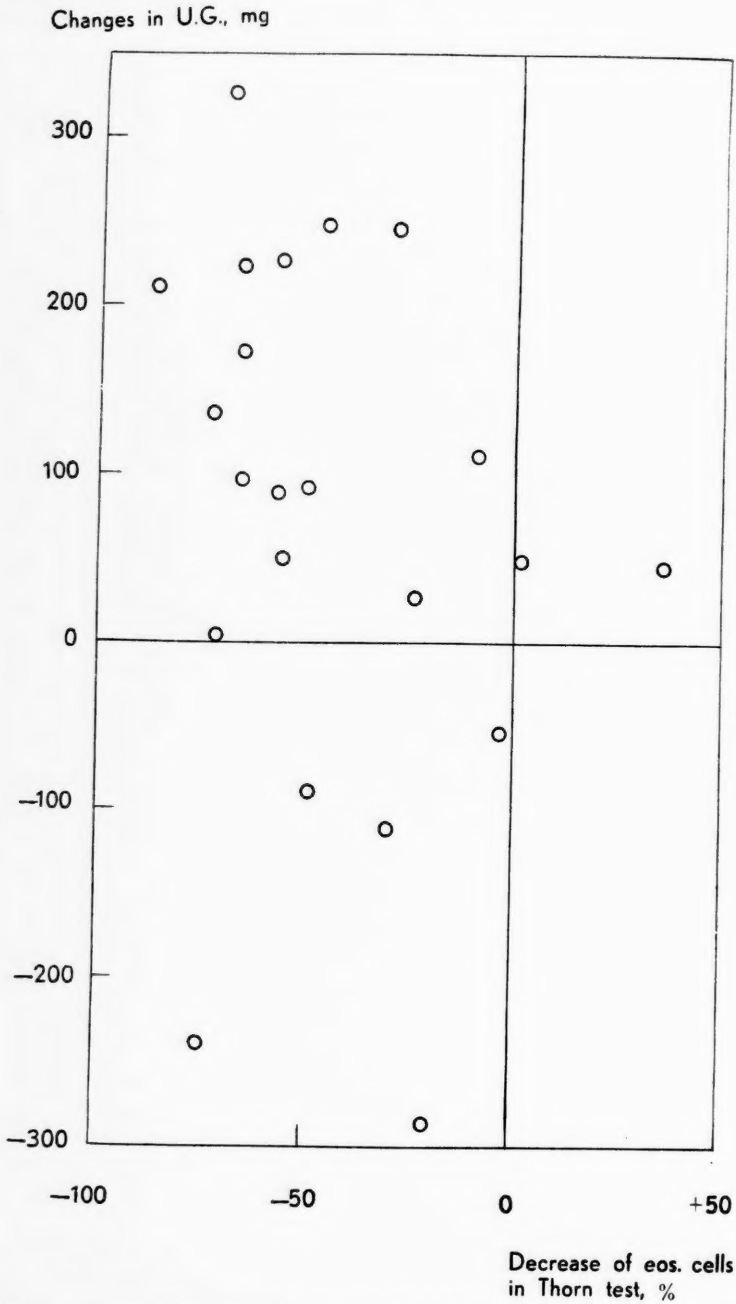


Fig. 17. — No graphic correlation was revealed between the average U.G. change from control days to ACTH or Cortisone days and the results of the Thorn test.

Spondylarthritis ankylopoietica

Urinary glucuronide determinations were made in 19 cases, in all of them on two successive days. The patient's age ranged from 20 to 70 years, average 39.1. All of them were men. The 24-hour mean urinary glucuronide value of the series was 478 mg (S.E. 29, standard deviation (S.D.) 126). This was a higher figure than the 24-hour value (444 mg) for the healthy control men. The difference between these results was not statistically significant ($t = 1.05$, $P = 0.1$). The mean urinary glucuronide value for men with rheumatoid arthritis was 398 mg; the corresponding mean for men with spondylarthritis was higher and the difference statistically significant ($t = 2.69$, $P = 0.01$). The disease had lasted less than 5 years in 7 of the 19 cases and over 5 years in the other 12. The mean urinary glucuronide value for the former group was 497 mg and for the latter 467 mg. The erythrocyte sedimentation rate of 8 patients was under 50 mm/hour, of 11 over 50 mm/hour. For the former group the mean urinary glucuronide value was 451 and for the latter 497 mg. The duration of the illness seemed to have a slight reducing tendency on the glucuronide values. In the under 50 mm/hour group the urinary glucuronide values seemed to be lower than in the other group.

It has been shown that high urinary glucuronide values seem to appear in some cases of spondylarthritis ankylopoietica. (Brummer, Savola and Leikkola 1957).

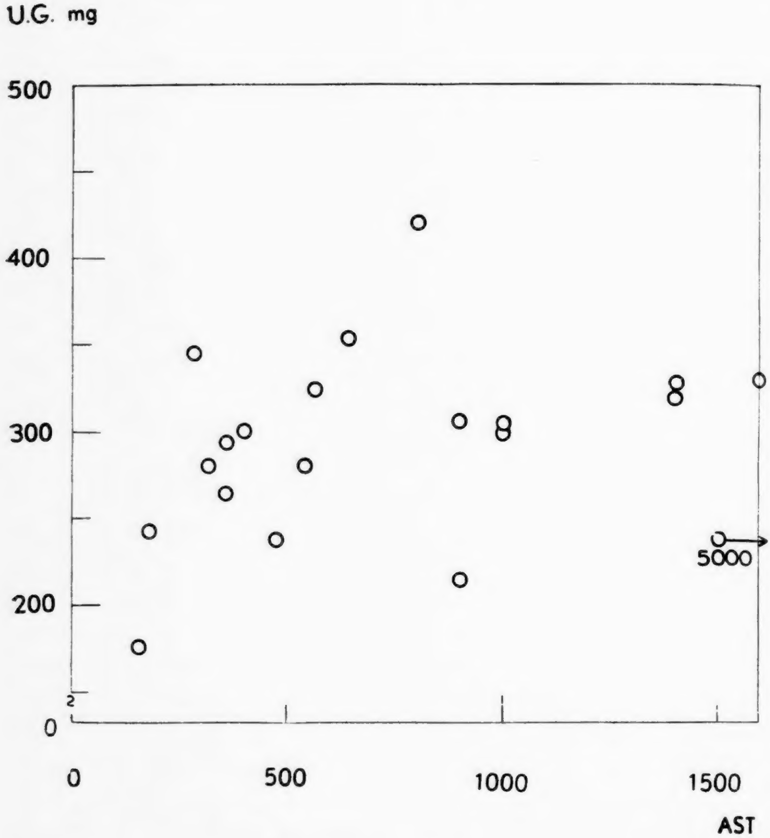


Fig. 18. — Urinary glucuronides (U. G.) and antistreptolysin titer (AST) in rheumatic fever material. There seems to be no correlation between U. G. and AST.

Rheumatic fever

The urinary glucuronide values of 20 patients were determined on two successive days. 16 of them were women and 4 men. The 24-hour mean urinary glucuronide value was 293 mg (S.E. 12, S.D. 55). As the corresponding mean for healthy persons was 427 mg, the quantity of urinary glucuronides was lowered in rheumatic fever and the difference was statistically significant (t = 8.34 and P = 0.001). The mean glucuronide value for men was 333 mg, for women 283 mg.

As Fig. 18 shows, there seemed to be no correlation between the antistreptolysin titre (AST) and the urinary glucuronide values of the series.

The series comprised 12 febrile cases with a mean urinary glucuronide value of 274 mg and 8 other cases with a corresponding mean value of 321 mg. The means of the leucocyte counts and sedimentation rate for the former group were c. 10,200 and 73 mm/hour, both higher than the corresponding figures for the latter group, viz. 8,700 and 49 mm/hour. Lower glucuronide values were demonstrated in the cases with active rheumatic fever than in the inactive cases.

Disseminated lupus erythematosus

The series comprised 10 cases whose blood showed the presence of typical L.E. cells (Zimmer and Hargraves 1952, Dubois 1953, 1956).

Seven of the patients were women and 3 men. (The series included a patient with hypertonia who had been given for a couple of years large doses of apresolin. The mean also included the determinations of 4 days made on a patient at different times of the year).

The 24-hour mean urinary glucuronide value was 291 mg. (S.E. 21, S.D. 70); compared with the corresponding mean for healthy persons, 427 mg, the difference was statistically significant ($t = 8.52$, $P = 0.001$). The quantity of urinary glucuronides thus seemed to have decreased in disseminated lupus erythematosus.

In 5 patients the disease had lasted 3 years or less and in 5 over 3 years. The means of these groups were 283 and 330 mg, respectively. Lower glucuronide values are possibly registered at the beginning of the disease than later. The mean sedimentation rate was high in both groups (in the former 90 mm/hour and in the latter 92 mm/hour); the cases were generally febrile. (The series however, was small.)

Some other diffuse collagen diseases and diseases affecting the joints

The series consisted of 12 cases which formed a heterogenous group of various diseases affecting the joints.

In a case of periarteritis nodosa (P.A.D.) the 24-hour urinary glucuronide values were 249 and 279 mg. As the case involved a male patient the values were distinctly low in comparison with the corresponding normal cases.

In 3 scleroderma patients the 24-hour values ranged between 297 and 421 mg. All the cases were women and on comparison with normal cases there seemed to be a tendency towards lower values.

In 2 patients with fibrositis, both of them women, the urinary glucuronide values ranged between 269 and 379 mg, slightly lowered. In 2 patients with periarthritis humeroscapularis and one patient with spondylarthrosis deformans no difference was observed in comparison with the urinary glucuronide values of healthy persons.

All the 3 cases of gonorrheal arthritis were women with glucuronide values between 267 and 413 mg. A distinct decrease in the quantity of glucuronides was observed in the active case (No. 275: febrile; sedimentation rate considerably elevated) but the change was very slight in the others. (Case No. 273 showed ankylosis and possibly arthritic deformation of the joint after arthritis gonorrhoica).

VII. DISCUSSION

Most investigators of glucuronide metabolism have determined only the urinary glucuronic acid excretion and the urinary glucuronides have been studied but rarely (Fishman and Green 1955, Brummer, Savola and Leikkola 1957). In the present investigation the glucuronides were determined as this value, methodically, is a more reliable indicator than the glucuronic acid values. In the former, oxidation with alkaline iodine solution removes compounds possessing an aldehyde group, e.g. pentoses, hexoses, etc.

The results showed the men in the control group to have higher 24-hour glucuronide values than the women; the difference was statistically significant. No significant differences were observed in the glucuronide values between the different age groups in either sex. It is interesting to find glucuronide fluctuations during the menstrual cycle in healthy women. A statistically significant increase in the glucuronide values around the middle of the menstrual cycle and a week after it compared with the values registered immediately before and after menstruation was established in the female control material.

In rheumatoid arthritis urinary glucuronide values were lower in both men and women in comparison with the corresponding values for the control groups. The differences were statistically significant. No significant differences were observed between the different age groups. During the menstrual cycle, female cases of rheumatoid arthritis showed similar variations to the control material. No significant difference was observed in the glucuronide values of the pre- and post-climacteric groups. As the disease became more active, rheumatoid arthritis showed a tendency to lover urinary glucuronide values and supporting this observation there were significant regressions between urinary glucuronide values and (only in women) fever and hemoglobin, and (in both sexes) the erythrocyte sedimentation rate,

electrophoretic albumin fraction and mucoproteins. Glucuronide values also seemed to decline as the degree of severity of the disease increased and the patient's functional capacity was impaired. The differences were not always statistically significant. No statistically significant differences were observed between the patients receiving gold therapy and the other patients with rheumatoid arthritis. Three-day administration of ACTH and cortisone in rheumatoid arthritis increased the excretion of urinary glucuronides compared with the excretion on control days; the difference was statistically significant.

In patients with ankylosing spondylitis glucuronide excretion seemed to increase a little compared with the control group though the difference was not statistically significant. The difference between the mean urinary glucuronide values in the ankylosing spondylitis group and the corresponding rheumatoid arthritis group was statistically significant. This observation is interesting from the theoretical standpoint and serves as additional proof that these diseases are different entities. The difference observed, however, has no significance worth always mentioning for differential diagnosis in special cases.

In the rheumatic fever and disseminated lupus erythematosus cases the quantity of urinary glucuronides decreased compared with the control groups; the differences were statistically significant.

The urinary glucuronide values of some patients with scleroderma, fibrositis, periarteritis nodosa and gonorrheal arthritis seemed to be slightly lower than those of the control material. No noteworthy difference from the controls was observed in a few cases of periarthritis humeroscapularis and arthrosis deformans.

The urinary glucuronide variations during the menstrual cycle were examined by determining the glucuronidates soluble in butyl alcohol (G.B.S.) and acetone (P.G.) in the urine during the menstrual cycle. It was found that both the fractions rose during the luteal phase to almost double the values during the follicular phases (G.B.S. = 30 mg $\pm$ 4 and 15 mg $\pm$ 4, whereas P.G. 13 mg $\pm$ 3 and 5 mg $\pm$ 2) (Jayle, Grepy and Meslin 1949). Similar results were reported by Bisset, Brooksbank and Haslewood (1949) from the variations of pregnanediol-resembling glucuronosidates during the menstrual cycle. These fluctuations occur coincidently with the variations in total urinary glucuronides observed in this research. The variations in quantity of G.B.S. and P.G., however, do not account for more than a small part of the total glucuronide fluctuations (e.g. the difference

between the total glucuronide means registered immediately after menstruation and around the middle of the menstrual cycle was 103 mg). No notable variations were observed in the β-glucuronidase activity in the blood during the menstrual cycle of healthy women, but the vaginal fluid gave the lowest values around the middle of the cycle (Fishman et al. 1951) when the β-glucuronidase activity of the endometrium of the uterus was highest (Odell and Fishman 1950). The above observations may be of interest when the role of β-glucuronidase activity in the glucuronide synthesis mechanism of the organism is explained.

The correlation between the exogenous glucuronide synthesis and glucuronide formation in the connective tissue is still an unsolved problem. In searching for an explanation for the low urinary glucuronide values in rheumatoid arthritis, however, it will be as well to examine the presence of substances occurring in the UDP (uridindiphosphoric) cycle (Fig. 2) in this disease. After determining the adenosine triphosphoric acid values in blood, Lövgren and Luksep (1952) distributed their rheumatoid arthritis cases into two groups. The low values could be ascribed to the process of healing with grave bone and cartilage destruction, while the high values occurred at the active stage of the illness at which the periarthritic processes were still dominating. Men showed in the present investication a tendency towards lower urinary glucuronide values as the degree of severity of the disease increased. The differences were not statistically significant. Women showed a slight difference statistically in groups III and IV; bone and cartilage destruction as well as low ATP values especially occurred in the more severe cases. The quantity of urinary glucuronides seemed to decrease in the active cases of rheumatoid arthritis while Lövgren and Luksep (1952) found high ATP values in these cases.

UDP-glucose has an important role (Fig. 2) in the UDP-cycle and the association with carbohydrate metabolism is a close one. The lowering of the carbohydrate tolerance in cases of rheumatoid arthritis was established in 1920 by Pemberton and Foster, but the same phenomenon occurred also in other infections. The drop in the citric acid content of the blood was statistically significant in rheumatoid arthritis compared with normal cases; the increase in the pyruvic acid values was not significant compared with the control values (Lövgren 1945).

The explanation for the increase in the pyruvic acid content of the blood might lie in the metabolism of the rheumatic tissue. Considerable metabolic changes have been observed in synovial membranes in connection with a rheumatic disease. The tissue reaction of a disease involves a considerable oxidative metabolism as well as elevated glycolysis. A considerable increase has been established in synovial membranes in the utilisation of glucose and in the production of lactate in comparison with normal cases. The metabolic activity of a rheumatoid synovium seemed to be associated with the local proliferative activity of the disease process (Dingle and Thomas 1956). No significant difference compared with normal was demonstrated in the lactic acid values of the blood in rheumatoid arthritis cases before and after muscular exercise although there was a slight tendency to low values (Kalliomäki, Pulkkinen and Savola 1957). As rheumatic tissue seems to show abundant lactate production and no elevated lactic acid values can be established in the blood, lactic acid may in oxidative conditions become oxidized to pyruvic acid. The lactic acid dehydrogenase of the enzyme catalysing this reaction (prosthetic group DPN) has been found in some cases of rheumatoid arthritis to be elevated in comparison with the controls (Hsich and Blumenthal 1956).

Many investigations concerned with carbohydrate metabolism and the metabolism of the connective tissue in rheumatoid arthritis thus either demonstrate or indicate the elevation of the pyruvic acid content of the blood. The phenomenon could support the thesis that low urinary glucuronic values occur in rheumatic diseases; the same applies to the evidence from animal tests that glucuronic acid excretion drops after the administration of pyruvic acid (Martin and Stenzel 1944).

Another explanation for the decrease in the quantity of urinary glucuronides would be the role of the liver. Lövgren (1945) found changes in the liver in over 50 % of an autopsy series of rheumatoid arthritis. Results liver biopsy also show liver damage (Lövgren 1953, Movitt and Davis 1953). Differences from normal cases have been reported in liver function tests (Rawls et al. 1937, 1939, Robinson 1943, Poulsen 1949, Laine et al. 1955). In the present investigation urinary glucuronide values showed a tendency to fall with the elevation of bromsulphalein test values. The correlation was not statistically significant.

Glucuronide formation occurs in the kidneys an to a significant degree in the intestines as well as in the liver (Hartiala 1954, 1955, Shirai and Ohkubo 1954). It is still difficult to decide the extent to which intestinal conjugations participate in urinary glucuronide formation in these investigation conditions. According to Hartiala (1957), the glucuronate conjugation of the intestines is associated primarily with the absorption processes in the intestine and probably affects urinary glucuronide excretion only when glucuronosiding substances have been administered. This, however, was avoided in the present study.

Renal lesions are not rare in rheumatoid arthritis (Teilum and Lindahl 1954 etc.) nor in rheumatic fever or disseminated lupus erythematosus. The role of this factor must be taken into consideration when trying to account for low urinary glucuronide values as glucuronide excretion might well be expected to decline when renal function is impaired.

VIII. SUMMARY

24-hour urinary glucuronide values were determined by the naphthorecorcin colour method of Fishman and Green (1955) in 73 healthy persons and 202 patients with a rheumatic disease or some other disease affecting the joints. A total of 788 duplicate determinations were made. The number of glucuronid acid determinations was 35. The statistical urinary glucuronide values were calculated from the 48-hour glucuronide means.

The mean 24-hour urinary glucuronide value of 71 healthy persons was 427 mg (S.E. 10). The corresponding value of 38 healthy men was 444 mg (S.E. 11) and of 33 healthy women 407 mg (S.E. 12). No significant differences in the glucuronide values were observed between the different age groups in either sex.

Urinary glucuronide values were observed in 14 healthy women during the inter-menstrual period. The values were statistically significantly lower immediately before and after menstruation than around the middle and a week after the menstrual cycle. Female rheumatoid arthritis patients showed during the menstrual cycle a similar fluctuation to healthy persons.

The 24-hour mean urinary glucuronide value of 53 men with rheumatoid arthritis was 398 mg (S.E. 7) and of 77 women 356 mg (S.E. 7). Compared with the corresponding control groups, the difference was statistically significant for both sexes. Increase in the activeness of rheumatoid arthritis was accompanied by a tendency towards lower urinary glucuronide values. In febrile women the urinary glucuronide values were lower than in fever-free women; the difference was statistically significant. This finding was not made with men. The statistically significant regressions occurring in rheumatoid arthritis between the urinary glucuronide values and the erythrocyte sedimentation rate, paper electrophoretic albumin fraction

of serum and serum mucoprotein values indicated that the quantity of urinary glucuronides falls as the disease becomes more active. A significant fall in glucuronide values in women but not in men accompanied impaired functional capacity and lower hemoglobin values. Neither the duration of rheumatoid arthritis nor the degree of the severity of the disease had any significant effect on the quantity of urinary glucuronides. No significant correlation was observed between glucuronides and total proteins, other paper electrophoretic fractions and the bromsulphalein test values.

The mean (478 mg, S.E. 29) urinary glucuronide value was slightly elevated in 19 cases of spondylarthritis ankylopoietica. The cases were all men. The difference was not statistically significant compared with the values of the control persons, but the difference was significant compared with the mean of the corresponding rheumatoid arthritis group.

In 20 rheumatic fever and 10 disseminated lupus erythematosus cases the fall in glucuronide excretion was statistically significant compared with the controls.

No significant difference was observed in rheumatoid arthritis in the urinary glucuronides values between patients given gold therapy and the other cases. In 33 cases of rheumatoid arthritis the administration of ACTH and cortisone for 3 days elevated the urinary glucuronide values in comparison with the values on control days before and after the administration of the drugs. The differences were statistically significant.

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